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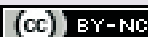
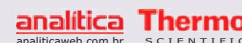
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Protein turnover, amino acid requirements and recommendations for athletes and active populations

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Abstract

Skeletal muscle is the major deposit of protein molecules. As for any cell or tissue, total muscle protein reflects a dynamic turnover between net protein synthesis and degradation. Noninvasive and invasive techniques have been applied to determine amino acid catabolism and muscle protein building at rest, during exercise and during the recovery period after a single experiment or training sessions. Stable isotopic tracers (¹³C-lysine, ¹⁵N-glycine, ²H₅-phenylalanine) and arteriovenous differences have been used in studies of skeletal muscle and collagen tissues under resting and exercise conditions. There are different fractional synthesis rates in skeletal muscle and tendon tissues, but there is no major difference between collagen and myofibrillar protein synthesis. Strenuous exercise provokes increased proteolysis and decreased protein synthesis, the opposite occurring during the recovery period. Individuals who exercise respond differently when resistance and endurance types of contractions are compared. Endurance exercise induces a greater oxidative capacity (enzymes) compared to resistance exercise, which induces fiber hypertrophy (myofibrils). Nitrogen balance (difference between protein intake and protein degradation) for athletes is usually balanced when the intake of protein reaches 1.2 g·kg⁻¹·day⁻¹ compared to 0.8 g·kg⁻¹·day⁻¹ in resting individuals. Muscular activities promote a cascade of signals leading to the stimulation of eukaryotic initiation of myofibrillar protein synthesis. As suggested in several publications, a bolus of 15-20 g protein (from skimmed milk or whey proteins) and carbohydrate (± 30 g maltodextrine) drinks is needed immediately after stopping exercise to stimulate muscle protein and tendon collagen turnover within 1 h.

Key words: Protein metabolism; Synthesis; Supplementation; Essential amino acids

General view of protein metabolism

In human beings, the body protein mass provides architectural support, enzymes to catalyze metabolic reactions, signaling intermediates within and between cell tissues, and fuel to support survival under extreme situations. Skeletal muscles are the major deposit of protein molecules (about 40% of body weight in young males with a 20-22 body mass index), and nearly 60% of total body protein in humans. Other organs or tissues contain protein, such as the liver, which synthesizes plasma proteins (including albumin, which represents nearly 50% of liver proteins), immune cells (mainly leukocytes), digestive enzymes, bone, and dermal collagen (1). For any cell or tissue, protein balance reflects the net protein synthesis and protein degradation that differ significantly among tissues and

organs and between cell compartments.

As there is no protein storage pool, the human body faces a delicate and dynamic balance that maintains homeostasis in the presence of environmental challenges. Under resting conditions, steady-state measurements of fuel turnover in postabsorptive humans show a unique situation where, as compared to carbohydrates and triglycerides, proteins have the fastest turnover rate and the lowest oxidation rate (Figure 1) (2).

Catabolic reactions and oxidation

The liver and, to a lesser extent, the kidney are the

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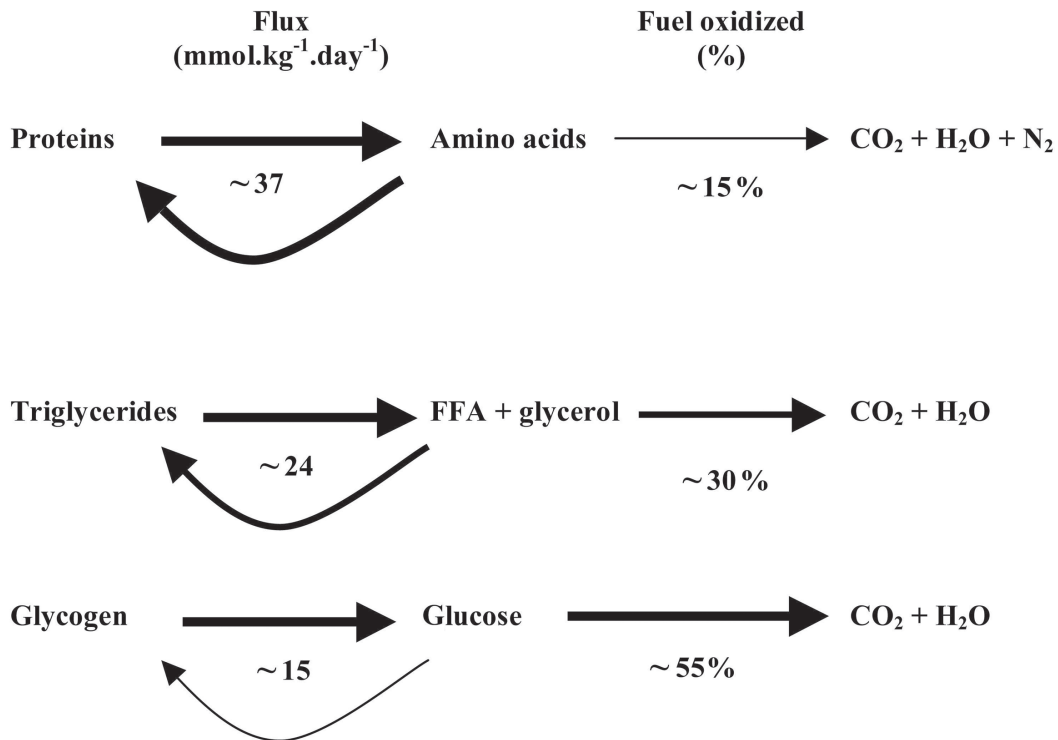


Figure 1. Rates of daily turnover and relative fuel oxidation in resting adults. FFA = Free fatty acids. Adapted from Ref. 1.

principal sites of amino acid metabolism in humans. When mammals are ingesting excess protein, amounts of amino acids larger than needed for synthesis of proteins and other nitrogen compounds cannot be stored or excreted and the surplus is oxidized or converted to carbohydrate and lipid. During amino acid degradation, the α -amino group is removed and the resulting carbon skeleton is converted into a major metabolic intermediate. Most of the carbon skeleton from amino acids is metabolized into pyruvate, acetyl-CoA or one of the intermediates of the tricarboxylic acid cycle (3).

The loss of the α -amino group occurs by oxidative deamination (using the enzyme glutamate dehydrogenase) and transdeamination (using several aminotransferases and glutamate dehydrogenase). Most of the amino acids can be converted to their respective oxoacids by reactions with aminotransferases (also called transaminases). All but two (lysine and threonine) amino acids appear to be able to be transaminated although it is not always clear how large a part these reactions play in the normal degradation of amino acids in the liver.

The reactions catalyzed by the aminotransferases (using pyridoxal phosphate-vitamin B6 as a prosthetic group) and by glutamate dehydrogenase (using NAD⁺ or NADP⁺ as oxidizing agents) are close to equilibrium so that 2-oxoacids are provided, the overall process can be readily

reversed and amino acids can be synthesized as well as degraded. The near-equilibrium transdeamination system provides an easy mechanism whereby the concentrations of both amino acids and 2-oxoacids are maintained fairly constant despite variations in the magnitude and direction of the metabolic flux through this system. The metabolism of amino acids, in addition to adenosine, generates most of the ammonia. Meanwhile, most tissues release nitrogen mainly as alanine or glutamine in order to buffer the toxicity of ammonia. The first reaction uses aminotransferase from glutamate to pyruvate, and the second reaction transfers the ammonia itself to glutamate and is catalyzed by glutamine synthetase.

Although a large proportion of the ammonia does not arise from catabolism in the liver, the urea cycle occurs exclusively in hepatic tissue and requires four molecules of "energy-rich" phosphate for the synthesis of one molecule of urea. In the human being, as much as 90% of urinary nitrogen is in the form of urea.

The urea cycle appears to be regulated by non-equilibrium reactions, with the first reaction being the flux-generating step. The synthesis of fumarate by the urea cycle is important because it links the urea cycle and the tricarboxylic acid cycle. In this respect, fumarate leading to oxaloacetate can be converted to glucose by a gluconeogenesis pathway.

Many cells are capable of concentrating amino acids

from the extracellular environment, but prior to intracellular metabolism, amino acids must be transported across the cell membrane. This transport is mediated by specific amino acid transporters, proteins that recognize, bind and transport these amino acids from the extracellular medium into the cell, or vice versa.

The skeletal muscles, the intestines and the liver are particularly important for the disposal of excess amino acids. Much of the nitrogen is channeled into only a few compounds for transport between the tissues (mainly alanine and glutamine). Free amino acid deposition in muscle often accounts for as much as 80% of the total amount in the whole body. In contrast, plasma contains a very small proportion of the total amino acid pool, ranging from 0.2 to 6% for individual amino acids.

The muscle free amino acid pool in a normal male weighing 70 kg has been calculated to be about 86.5 g excluding taurine and 121.5 g including taurine (3). The latter compound is synthesized from cysteine, and taurine is excreted as such or in the form of taurocholate or related bile salts. In mammalian muscle tissues, taurine acts as a membrane stabilizer and a modulator of the Ca^{2+} storage capacity of the sarcoplasmic reticulum. Of the total pool of human skeletal muscle free amino acids, the eight essential amino acids (EAA) represent only 8.4%, whereas glutamine, glutamate, and alanine constitute nearly 79%. Among the amino acids, the branched-chain amino acids (leucine, isoleucine, and valine) are of particular interest as 60% of the total distribution of specific enzymes necessary for their oxidation (α -keto acid dehydrogenases) in humans is located in skeletal muscle. These amino acids, unlike most of the others, are taken up by the striated muscles after a meal and partially oxidized in those tissues. In the postabsorptive period of starvation, the human leg muscle essentially releases alanine and glutamine (60% of the total release).

Amino acid oxidation in muscle leads to an appreciable amount of ATP generation. Assuming a common composition for the amino acids in muscle, the total balance of amino acid degradation is: 1 mol amino acids + 5 mol $\text{O}_2 \rightarrow 0.70$ mol urea + 4.11 mol CO_2 + 0.34 mol SO_4^{2-} + 22.19 mol ATP. This equation also states that about 0.4 mol equivalent of glucose can be formed in the liver, or 72 g from the 110 g of mixed amino acids. The complete oxidation of leucine, isoleucine and valine yields 43, 42, and 32 mol ATP, respectively, per amino acid molecule. However, the P/O ratio is only 2.4 compared to 2.8 for fats and 3.1 for glycogen; thus, amino acids are not a good fuel for maximum power production.

At present, it is rather difficult to precisely estimate the energy balance from the daily supply of amino acids in muscle tissue, especially in humans. However, ~90% of the nitrogen derived from the branched-chain amino acids (BCAA) is released as glutamine and only ~10% as alanine. Jungas et al. (4) calculated the net ATP and acid-base

balances associated with amino acid oxidation in skeletal muscle and concluded that, under resting conditions, the overall ATP balance amounts to ~4.5 mol excess ATP per day, or about 50% of the total oxidation from amino acids in muscle, small intestine, kidney and liver tissues taken together. Again, this emphasizes the importance of muscle mass in the energy balance of the whole organism. The net effect of the oxidation of amino acids to glucose in the liver is to make nearly two-thirds of the total energy available from the oxidation of amino acids accessible to peripheral tissues.

Methodology for protein turnover studies

Our scientific heritage in the study of protein metabolism started with Black (1756) and Rutherford (1772) who observed the release of CO_2 and NH_3 in animal tissues, respectively. Since then, the metabolic changes produced by an intake of protein have been widely studied. Currently, methodological approaches are based on the dilution of an isotope tracer and its application to the calculation of the synthesis and turnover of whole-body and tissue proteins.

Mammalian organisms use a considerable amount of energy in the basal or standard state when no net work is being done and all free energy is dissipated. Proteins, polymers of amino acids, are continuously degraded (catabolism) and renewed (anabolism) through numerous and complex metabolic processes. Standard metabolic rate may be defined as the steady-state rate of heat production by the whole organism under "standard" conditions (awake and resting).

Direct measurement of protein synthesis can provide relevant information regarding the metabolic state of individual tissues. Direct incorporation methods using tracer molecules have been used to measure the synthesis of specific proteins. The principal method is to provide a known amount of a labeled compound (tracer) to be mixed with the endogenous pool of amino acids that is to be incorporated into protein over time. Several techniques, both noninvasive and invasive, have been used to analyze human protein synthesis and breakdown during and after exercise.

Noninvasive techniques

$^2\text{H}_2\text{O}$ administration. Labeled water is administered orally to the subjects (about 5 mL/kg body weight) (5). The di-deutero-labeled water is rapidly dissociated to yield mono-isotopically labeled water (^2HHO), and the equilibrium of ^2H -labeled water with various amino acids leads to the generation of singly labeled compounds. The uniqueness of using $^2\text{H}_2\text{O}$ is that, unlike amino acids, which must gain entry into the cells via transporters, ^2H -labeling occurs intracellularly (Figure 2) (5).

A few studies of the effects of feeding and exercise on muscle protein synthesis (MPS) have been conducted in mice, rats and human males (5).

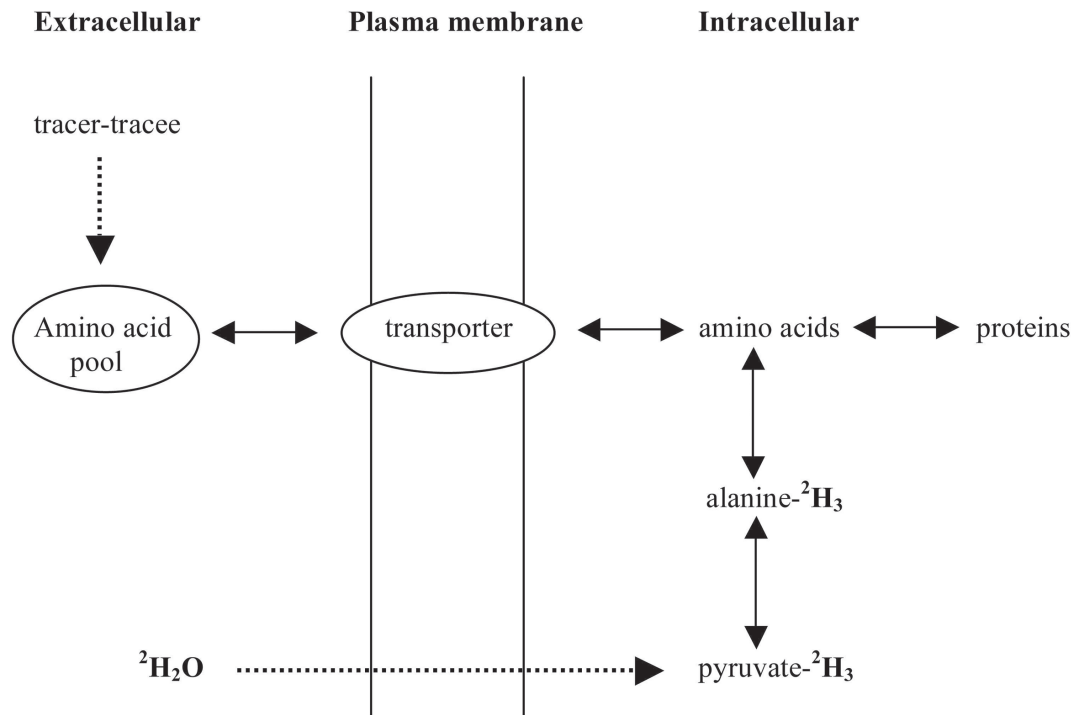


Figure 2. Precursor-product labeling methods to determine tissue protein synthesis. Adapted from Ref. 5.

Whole-body nitrogen balance. For many years the whole-body nitrogen balance (NBal) has been the easiest and fastest way to estimate the amount of protein intake needed to cover the daily requirement of protein in a resting human subject. The NBal was a simple equation: nitrogen content of food intake (expressed as mg N from proteins) - total nitrogen excretion (urine, feces, sweat). For practical reasons, the latter part of the equation was restricted to urine N, with some correction from the two other origins. Thus, NBal = 0 means an accurate daily protein intake. Moreover, specifically the estimation of an adjusted intake was obtained by comparison of the NBal values with the daily intake of protein expressed as dry g/kg body weight. The Institute of Medicine (USA) proposes a daily intake of 0.8 g/kg to cover the total protein metabolism in resting adults (6).

Despite the fact that urinary nitrogen is a validated biomarker for dietary protein intake, there are several uncertainties such as the validity of dietary assessments, underreporting behaviors, measurement errors, and incomplete 24-h urine collection (7). Usually, several days (a whole week of 7 continuous days) of recording intake and at least two 24-h urine collections are needed to minimize these biases. Completion of 24-h urine collection may be validated with para-aminobenzoic acid (PABA), which is actively absorbed and excreted in urine. The method consists of three 80-mg tablets of PABA taken with meals.

Urine collections that contains $\geq 85\%$ PABA can be classified as satisfactory. Additionally, this NBal method is rather inexpensive compared to isotopic methods.

Invasive isotopic tracer methods to quantify protein turnover

Radioactive (³H, ¹⁴C, ¹⁸N, and ¹¹O) and stable isotopes (²H, ¹³C, ¹⁵N, and ¹⁸O) have been used to study metabolism in biological circumstances. The advantages and disadvantages of the use of stable isotopes as biological tracers were analyzed by Halliday and Rennie (7). The major advantage of the use of stable isotopes is the lack of ionizing radiation, being therefore safer and less toxic for humans. Their disadvantages are the low range of measurement of stable isotopes (less than 1%) as compared to stable elements, and the high costs of chemical compounds and instrumentation. These investigators noted that the enrichment of muscle protein with [1-¹³C]-leucine using both isotope ratio and gas chromatograph/mass spectrometer techniques permitted the measurement of MPS during a total investigation time of about 7 h. Whole-body steady-state tracer techniques assume a simple two-pool model for protein metabolism in which amino acids are either free or protein bound. But there are more invasive methods that involve arteriovenous catheterizations. Moreover, a recent publication by Smith et al. (8) reviewed different tracer methods to evaluate human muscle protein turnover and the authors concluded

that the variability of the reported values is due in part to 1) differences in experimental design (choice of tracer) and 2) considerable within-subject variability. The lack of consensus reflects the fact that measurement of human protein metabolism *in vivo* has inherently difficult problems to solve (inaccessible compartments, metabolically different compartments).

Measurements of whole-body protein turnover from the dilution of tracer amino acids in plasma. Stable isotopic tracers are administered as a priming dose and then continuous intravenous infusion: ^{15}N -glycine, ^{13}C -lysine, $^2\text{H}_5$ -phenylalanine. Nowadays, the ^{15}N -glycine method has been gradually replaced with ^{13}C -lysine and $^2\text{H}_5$ -phenylalanine. Isotopic enrichment at isotopic steady state is analyzed from a few blood samples (see Ref. 9). Over the years, ^{13}C -lysine has become the reference method as a tracer, and the isotopic enrichment of its transamination product, α -ketoisocaproic acid, is used as an estimate of the intracellular isotopic dilution of the leucine pool.

Approaches to the measurements of tissue protein synthesis using arteriovenous differences. Stable isotopically labeled tracers have been used to evaluate MPS. This specific technique needs arteriovenous differences and muscle biopsy samples. The low isotope enrichments of ^{13}C -lysine and $^2\text{H}_5$ -phenylalanine perfusions are measured by a combination of gas chromatography and mass spectrometry giving access to MPS and balance (10). However, as observed in human muscle tissues, the metabolic fate and the fractional synthesis rate (FSR) depend on the choice of the amino acid tracers (1,11). At present, it is possible to evaluate the distinct muscle fractions (myofibrillar, sarcoplasmic, mitochondrial) using different separation techniques such as gel electrophoresis and cation-exchange chromatography (1,12). A new technique based on $[5,5,5\text{-}^2\text{H}_3]$ -leucine perfusion and SDS-PAGE separation is able to identify muscle fiber myosin heavy chain, and therefore to distinguish slow- and fast-twitch muscle fibers (13).

Jaleel et al. (14) reported a methodology based on $^{13}\text{C}_6$ -phenylalanine labeling and two-dimensional gel electrophoresis to measure synthesis rates of human multiple muscle mitochondrial proteins. The *in vivo* synthesis rate of individual skeletal muscle mitochondrial proteins in humans was also measured after SDS-PAGE of anion exchange HPLC extracts (15). Stable isotope techniques did study the incorporation of labeled amino acids (^{13}C -proline, ^{15}N -proline) into tendon and ligament to study the kinetics of human collagen (16,17).

Some more recent techniques for the measurement of tissue turnover have been used in humans to precisely identify specific muscle protein modifications during exercise, such as the mRNA activity of different contractile proteins. However, due to post-translational adaptations, it is difficult to quantitatively assume that the modified mRNA expression is true valuable evidence of specific protein synthesis.

Protein turnover in resting individuals

General view

The term turnover covers both the synthesis and breakdown of protein. In a steady-state condition, the energy cost of protein synthesis will account for approximately 10% of the basal oxygen uptake (18). Total protein synthesis in adult human subjects is about $3.0 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ while protein turnover is about $5.7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ (18). Protein degradation in human skeletal muscles estimated from the release of tyrosine in the presence of insulin and amino acids is approximately $34 \text{ nmol}\cdot\text{h}^{-1}\cdot\text{g wet weight}^{-1}$. This degradation rate corresponds to a half-life of approximately 20 days.

The optimal intakes of whole protein in the human diet have been a matter of debate for many years (see Ref. 6). The NBal data for healthy adult men and women increased from $0.60 \text{ g/kg per 24 h}$ in 1979 to $0.80 \text{ g/kg per 24 h}$ in 2003. However, the recommended intake of protein is higher for young children ($1.2 \text{ g/kg at 1 year}$) and slowly decreases for young adults (18 years). The energy cost of protein turnover in healthy elderly humans (60-75 years) is comparable to that of a young population, and correlates with fat-free mass (18,19).

Vegetarians restrict their diet to plant food and may be at risk of not getting adequate amounts of some indispensable amino acids (lysine, methionine, cysteine, threonine) because the amount of protein in plant foods compared to animal foods is inadequate. Moreover, plant proteins are generally less digestible than animal proteins. Nevertheless, available evidence does not support the recommendation of a separate protein requirement for vegetarians who consume complementary mixtures of plant protein (6).

We shall now focus our interest mainly on skeletal muscle and collagen (tendon) tissues, which appear to be quantitatively most important for physically active people.

Skeletal muscle tissues

Skeletal muscle tissue contains a few thousand specific proteins, which could be classified as myofibrillar, sarcoplasmic and mitochondrial fractions. The myofibrillar proteins are different molecules such as myosin heavy and light chains, actin, tropomyosin, troponins (T, I, and C), titin, elastin, etc. Sarcoplasmic proteins (which represent about 20-30% of total muscle proteins) consist of glycolytic enzymes, proteins of the sarcoplasmic reticulum (calsequestrin, calcium-ATPase, and others). Mitochondrial proteins in muscle tissue consist of enzymes of the tricarboxylic acid cycle, β -oxidation, and mitochondrial respiratory chain. The rate of myosin synthesis is lower than that of other muscle fractions (Table 1).

Thus, it appears that the FSR of actin is more or less 2-fold that of the myosin heavy chain. The precise mechanism of this specificity remains unknown. The overall control of the size of human skeletal muscle mass has been elegantly reviewed by Rennie et al. (20). Eventually,

Bohé et al. (21) concluded that the rates of synthesis of all classes of muscle proteins (mixed, myofibrillar, sarcoplasmic, mitochondrial) are acutely regulated by the blood essential amino acid concentration over their normal diurnal range, but become saturated at high concentrations (21). Thus, the stimulation of protein synthesis depends on the concentration of extracellular rather than intramuscular EAA.

Collagen tissues

Extracellular matrix located in tendon tissue ensures a functional link between the skeletal muscle mass and the bone. The extracellular matrix molecules consist of a variety of glycoproteins most of which consist of proteoglycans and collagen fibrils, the latter being predominant (60-85%) (see Ref. 22). The fundamental building blocks of collagen fibrils consist of three polypeptide α -chains that compose a triple helical structure. Collagen is 35% glycine, 21% proline and hydroxyproline, and 11% alanine. This unusual amino acid content is imposed by structural constraints unique to collagen molecules.

Collagen is the most abundant single protein in most vertebrates (humans included), constituting up to nearly one third of the total protein mass. Collagen molecules are not synthesized in muscle tissue but are synthesized in fibroblasts, which are scattered within the tissue. Given the importance of collagen to skeletal muscle function, the knowledge of its quantitative synthesis rate was either ignored or estimated to be very slow. Over the last few years, some studies have revealed that FSR of patellar tendon collagen amounted to a mean value of 1.08% per day in resting men, and nearly 30% less in women (23). When comparing the FSR of collagen and myofibrillar proteins in humans, there is no major difference as previously assumed, but muscle collagen is not at all responsive to feeding (24). Meanwhile, collagen synthesis is similar in muscle after eccentric and concentric exercise (25).

Growth hormone (GH) and recombinant human GH have no effect on human muscle size or MPS (26) but do have a positive effect on strengthening the collagen matrix in musculotendinous tissue (27).

Protein metabolism in exercising individuals

In 1974, Goldberg et al. (28) showed that the rate of protein degradation depends upon the level of muscular activity. Rennie et al. (29) provided further evidence that severe physical exercise by adult humans is accompanied by an increase in the rate of whole protein breakdown and a decrease in the rate of synthesis. More information about the development of data may be obtained from a review (30). The measurement of 3-methylhistidine (3-MeHis) in urine has been recognized as a useful, indirect, non-invasive technique to assess muscle protein breakdown (MPB). The major site of endogenous 3-MeHis is the actin

Table 1. Protein fractional synthesis rates (FSR) in human skeletal muscle (fasted state).

Muscle fractions	FSR (% per day)
Myosin heavy chain	0.90 \pm 0.08
Actin	1.80 \pm 0.19
Sarcoplasm	1.29 \pm 0.20
Mitochondria	1.94 \pm 0.10

Data are reported as means \pm SD. Adapted from Ref. 1.

of all muscle fibers and the myosin of white muscle fibers (see Ref. 30 for details). However, Rennie and Millward (31) believe that urinary 3-MeHis is not a valid index of skeletal muscle production of 3-MeHis. This is why researchers now use isotopic techniques to evaluate protein turnover in human beings and to distinguish the effects of resistance from endurance exercise and training in skeletal muscle fibers (23,32-34) and tendons (17,23,35). As stated by Kumar et al. (32), exercise is characterized as either "endurance/aerobic" or "resistance" types. The main operative distinction results in a phenotypic shift towards a population of fibers with greater oxidative capacity with endurance exercise, whereas repeated resistance exercise induces fiber hypertrophy. However, during exercise itself, both types of exercise depress MPS with unchanged MPB.

Effect of resistance exercise

There are a few review publications related to the regulation of human MPS and MPB during and after resistance exercise (32,36). We will have to differentiate the results obtained in the fasted (postabsorptive condition) or fed state, during or after exercise, for MPS or MPB, using different modes of amino acids or protein supplementation.

The data in Table 2 clearly demonstrate that short-term intense resistance exercises induce a greater increase in the amount of mixed skeletal muscle protein at the end of exercise. The differences between the reports may be the result of methodological differences (see "Invasive isotopic tracer methods to quantify protein turnover") but, generally speaking, the best results seem to be linked to the total work output (80-90% of maximal contraction). Resistance exercise seems to be more relevant regarding myofibrillar proteins than sarcoplasmic protein synthesis (37). Moreover, it appears that the increases in MPS persist up to 4 h after the end of exercise. On the contrary, resistance exercise does not seem to have a major effect on MPB.

The effect of feeding a mixed protein meal under resting conditions doubles MPS. Several publications have reported the results of resistance exercise on human MPS and breakdown (see Table 3).

Protein feeding has been applied after the end of ex-

ercise. Under most, if not all conditions, MPS has been enhanced by ingestion of amino acids. The increase in MPS is observed in mixed muscle, myofibrillar and sarcoplasmic fractions. The enhanced amount of muscle proteins depends on the quantity of the ingested amino acids, the relative proportion of EAA being either supplemented as free EAA or as a major portion of whey proteins (about 50% EAA). It appears that MPS is increased when the amino acids are ingested immediately after the end of the exercise session (38). Rapid aminoacidemia during the post-exercise period enhances MPS and the anabolic signals leading to the increase in muscle protein mass. Moreover, it seems that a bolus dose of 25 g is more efficient than a series of small pulsed drinks (10 x 2.5 g) (39). Both myofibrillar and sarcoplasmic proteins may remain stimulated up to 3-5 h post-exercise (38-40) or even up to 24 h in young men when the intensity of exercise is high (41).

Table 2. Effect of resistance exercise on total muscle protein synthesis (MPS) and muscle protein breakdown (MPB) under untrained conditions in a fasted state.

Exercise protocol	FSR (%/h) Post-Ex/Pre-Ex ratio	FBR (%/h) Post-Ex/Pre-Ex ratio	Reference
Mixed muscle proteins			
4 x 6-12 rep. 80% max	+49%*	-	105
5 x 10 rep. 100% max	+136%*	-	106
8 x 8 rep. 80% max	+140%*	+36%*	107
8 rep. 120% max	+122%*	+40%*	108
6 x 8 rep. 80% max	+30%*	-	109
8 x 10 rep. 75% max	+36%*	NS	110
10 x 10 rep. 80% max	+50%*	-	111
4 x 10 rep. 80% max	+135%*	-	112
5 rep. 90% max	+350%*	-	37
Myofibrillar proteins			
5 rep. 90% max	+330%*	-	37
Sarcoplasmic proteins			
5 rep. 90% max	+170%*	-	37

MPS and MPB are reported as their fractional synthetic rate (FSR) and fractional breakdown rate (FBR), respectively. rep. = repetitions; max = % max power output; Pre-Ex = pre-exercise; Post-Ex = post-exercise; (-) = not measured. *P < 0.05 compared to pre-exercise test. NS = no significant difference between pre- and post-exercise (P > 0.05).

Table 3. Effects of resistance exercise on human muscle protein synthesis (MPS) and muscle protein breakdown (MPB) in the fed state.

Exercise protocol	Feeding protocol	FSR (%/h) Post-Ex/Pre-Ex ratio	FBR (%/h) Post-Ex/Pre-Ex ratio	Reference
Mixed muscle proteins				
5 x 10 rep. 100% max	10 g AA (iv)	+121%*	NS	113
10 x 8 rep. 80% max	6 g EAA (oral)	+340%*	NS	114
4 x 10 rep. 80% max	10 g whey + CHO (oral)	+130%*	-	98
10 x 10 rep. 70% max	Leu EAA + CHO (oral)	+167%*	-	84
4 x 10 rep. 100% max	40 g egg proteins (oral)	+90%*	-	43
Myofibrillar proteins				
5 x 10 rep. 80% max	1 g protein/kg (oral)	+83%*	-	43
20 x 10 rep. 75% max	6 g protein/h (oral)	+188%*	NS	115
stepping ex (+25% bw)	45 g EAA + CHO	+221%*	-	116
5 x 10 rep. 100% max	25 g whey (oral)	+229%*	-	40
8 x 10 rep. 100% max	25 g whey (oral)	+193%*	-	39
10 x 8 rep. 80% max	0.3 g/kg LM whey	+90%*	-	38
Sarcoplasmic proteins				
20 x 10 rep. 75% max	6 g protein/h (oral)	+300%*	-	115
5 x 10 rep. 100% max	25 g whey (oral)	+104%*	-	40

MPS and MPB are reported as their fractional synthesis rate (FSR) and fractional breakdown rate (FBR), respectively. Pre-Ex = pre-exercise; Post-Ex = post-exercise; max = % max power output; AA = amino acids; EAA = essential amino acids; CHO = carbohydrate; LM = lean mass; (-) = not measured. *P < 0.05 (post-exercise compared to pre-exercise). NS = no significant difference between pre- and post-exercise.

Effect of endurance exercise

Again, it is necessary to differentiate between the effect of aerobic exercise under fasting or fed conditions. Table 4 summarizes the published data.

Endurance-type exercise seems to have less impact on skeletal MPS compared to resistance exercise. This may be due, at least in part, to post-exercise proteolysis of non-myofibrillar proteins. Indeed, prolonged aerobic exercise (>1 h) induces a higher release of nitrogen both in blood and urine (30). Apparently, MPS increases after the end of the event and not during prolonged exercise (42).

Effect of exercise training on muscle protein synthesis and breakdown in humans

According to Kumar et al. (32), it appears that chronic resistance exercise increases mean muscle fiber cross-sectional area and provokes muscle hypertrophy. Several investigators have reported an enhanced basal rate of MPS but it seems difficult to have a precise idea about these changes due to the lack of information on the time course of the last bout of exercise sessions during the training schedule. However, an accurate report before and after 10 weeks of training indicated an increase in the basal synthesis of myofibrillar proteins under resistance exercise, while endurance training enhanced basal mitochondrial protein

synthesis (43). Furthermore Meredith et al. (44) investigated the effect of different regimes of protein intake (0.6, 0.9, 1.2 g·kg⁻¹·day⁻¹) during endurance training in men and observed that the mean recorded NBal was equilibrated for 0.94 g·kg⁻¹·day⁻¹. Nevertheless, 11 of the 12 subjects had a positive balance of >1.2 g·kg⁻¹·day⁻¹.

Sex differences in muscle protein metabolism under exercise conditions

The literature does not provide significant information about a smaller muscle mass in women compared to men, unless anabolic hormonal injections (such as testosterone) are administered (45,46). Vingren et al. (47) speculated about the differential effects of several hormones, such as gonadotrophin-releasing hormone and adrenocorticotrophic hormone, which could explain the sex difference in muscle mass. They concluded that testosterone plays only a minor role in explaining the difference of muscle mass between women and men. Moreover, Kumar et al. (32) did not report differences in the basal or post-exercise rates of MPS or MPB between young men and young women. In addition, using two variable protein intakes, Pannemans et al. (48) observed identical nitrogen balance and whole-body protein turnover in young men and women. However, postmenopausal women have about 20-30% higher basal rates of

Table 4. Effect of endurance-type exercise on human muscle protein synthesis (MPS) and muscle protein breakdown (MPB) in the fasted and fed states.

Exercise protocol	Food protocol	FSR (%/h) Post-Ex/Pre-Ex ratio	FBR (%/h) Post-Ex/Pre-Ex ratio	Reference
Fasted state				
Mixed muscle proteins				
Swimming 1.5 h		+42%*	-	109
Swimming 2.7 h		+82%*	-	109
Running 45 min, 45% max		+80%*	NS	117
Cycling 1 h, 70% VO ₂ max		+22%*	-	54
Fed state				
Mixed muscle proteins				
Cycling 2 h		+48%*	-	118
Cycling 2 h, 55% Wmax		+84%*	-	91
Cycling 3 h, 55% Wmax	10 g protein/h + CHO 0.2 g protein·kg ⁻¹ ·h ⁻¹ 78 g protein + 234 g CHO	+51%*	-	42
Myofibrillar proteins				
Cycling 45 min, 75% max		NS	-	43
Leg ex. 1 h, 67% max	1.1 g protein/kg 1.4 BMR, 15% protein	+25%*	-	23

MPS and MPB are reported as their fractional synthesis rate (FSR) and fractional breakdown rate (FBR), respectively. Pre-Ex = pre-exercise; Post-Ex = post-exercise; % max = % max power output; CHO = carbohydrate; BMR = basal metabolic rate; Wmax = maximal power; (-) = not determined. *P < 0.05 between pre- and post-exercise. NS = no significant difference between pre- and post-exercise.

MPS than men (49). Thus, there is still a need for further investigation of the differential mechanisms.

Aging and muscle protein metabolism during exercise

There is some controversy regarding the rates of basal MPS in the elderly, with some investigators reporting reduced basal MPS rates compared to young men while others show that aging has no major effect on the basal rate of MPS in healthy men (see Ref. 32). However, resistance exercise training and appropriate nutrition can stimulate MPS, although with a 30% lower response in older men than in young men (50). Additionally, Haub et al. (51) observed that resistance-trained older men (65 ± 5 years) were able to increase their muscle strength and size when total protein intake was adequate (from 1.03 to $1.17 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$). There is almost no data about the evolution of MPB in response to exercise in elderly subjects. Nevertheless, it seems valuable and important to stimulate physical activity, with both resistance and endurance intervals, to adequately reduce the effect of aging on muscle metabolism.

Mechanisms leading to the regulation of muscle protein synthesis

MPS and degradation are regulated by hormonal and nutritional factors (2,20), which act on the sarcolemma receptors and sarcoplasmic effectors that promote the activation of translational initiation of protein synthesis.

Hormonal implications. Basically, four main hormones appear to be the major effectors acting on body protein metabolism: insulin, insulin-like growth factor-1 (IGF-1), testosterone, and GH. It is commonly reported that resistance exercise of moderate to high intensity and volume induces the blood release of IGF-1, testosterone and GH. However, as mentioned previously, the exact role of testosterone in resistance training programs is still hard to identify (47). But an elegant report by West et al. (52) reveals that even if transient resistance exercise induces increases in these anabolic hormones, they do not enhance post-exercise MPS. More recently, Phillips (53) estimated that anabolic hormone intervention in the adaptation of MPS after resistance exercise is more likely to be "chasing a hormonal ghost". Thus, other local intramuscular mechanisms appear to monitor the acute effect of the MPS response after resistance exercise.

Regulatory mechanisms of skeletal muscle protein turnover during exercise. Mechanical deformation of skeletal muscle fibers induced by muscle contraction stimulates several signals included in the sarcoplasm (2,32,33,43,54-57). Among the regulators acting on gene expression are amino acids, AMP-activated protein kinase (AMPK) (58), mitogen-activated protein kinase (MAPK) (37), the mammalian target of rapamycin complex 1 (mTORC1) (59,60), and the ribosomal S6 protein kinase 1 (S6K1) (61).

The EAA, mainly leucine (37,62,63) and glutamine (64),

the most abundant muscle amino acid, act on several kinases to stimulate the initiation of protein synthesis translation. A prominent signaling pathway that controls the regulatory process of protein synthesis involves the phosphorylation state of several regulatory proteins (AMPK, mTORC1, S6K1, MAPK) leading to increased myofibrillar protein synthesis after resistance exercise training and regulatory oxidative enzymes during endurance training.

A simplified schematic diagram is proposed in Figure 3A and B. Acute changes in protein synthesis are primarily regulated at the level of mRNA translation via translational efficiency (37,65) (Figure 3A). However, over the last few years, several publications have revealed that noncoding RNAs, called microRNAs (miRNAs), control the development, function and adaptation of skeletal muscle (66) through a post-transcriptional mechanism involving inhibition of translation and/or degradation of mRNA transcripts. Several studies show that exercise is capable of modulating miRNA levels, which play a central role in skeletal muscle plasticity (see Ref. 67).

The miRNAs are defined as 21-30 small single-stranded noncoding RNAs produced from hairpin-shaped precursors. From a microRNA gene, a primary-miRNA (pri-miRNA) is initially transcribed by RNA polymerase II in the nucleus as long primary transcripts of several kilobases. Then, an RNA II endonuclease cleaves the pri-miRNA into a 60-70 nucleotide (pre-miRNAs). An exportin-5-GTP transports the pre-miRNA from the nucleus to the sarcoplasm where it is cut by an RNA III enzyme into a 22 nucleotide mature miRNA. Skeletal and cardiac muscles are highly enriched in several miRNAs, named myomiR (miR). miR-206 is a unique member of the myomiR family in that it is specifically expressed in skeletal muscle. Kim et al. (68) suggested that miRNA-206 negatively regulates DNA polymerase translation, thereby inhibiting DNA synthesis. Thus, these myomiR could block the formation of skeletal muscle mass. Moreover, it is postulated that miR-206 has an important role in regulating the expression of genes involved in satellite cell specification during fiber type transitions in muscle.

A few reports on miRNA have been published recently on the effect of endurance exercise training in human subjects. Keller et al. (69) observed that endurance training down-regulates several miRNAs targeting DNA sequences involved in muscle differentiation (69). Additionally, Nielsen et al. (70) reported that several myomiRs are down-regulated between the end of the training period and two weeks of cessation. Linear correlations were observed between peak exercise levels of some blood circulating miR and VO_2max (71). Conversely, bed rest of only 7 days abolishes the gene responses involved in exercise adaptation, such as regulatory oxidative enzymes (72). However, there seems to be higher and lower responders to microRNA expression in skeletal muscle, which may explain that some subjects are less prone to react to resistance training than others (73). Finally, an important question remains: what is regu-

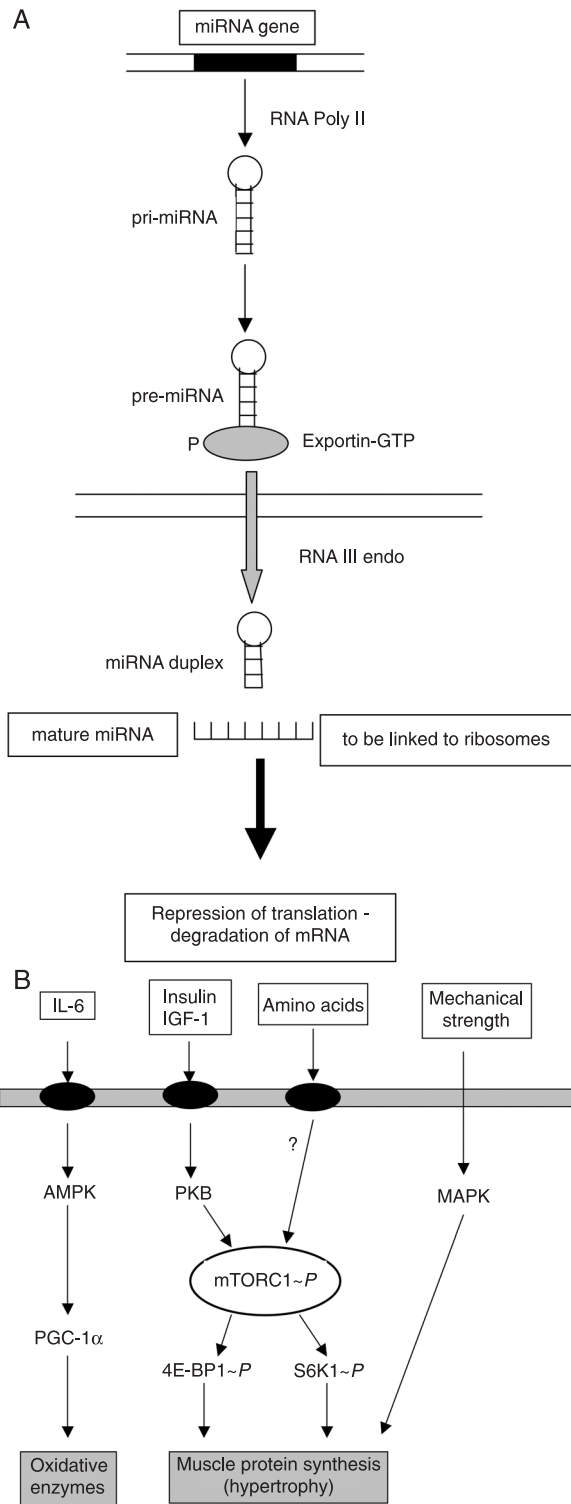


Figure 3. Simplified schematic diagram of skeletal muscle protein synthesis following exercise. **A**, The microRNA (miRNA) synthesis pathway (see text). **B**, The metabolic cascade from protein kinase B (PKB), mitogen-activated protein kinase (MAPK) to protein synthesis (see text).

lating myomiR transcription? The adaptation mechanism remains unknown!

After stimulation by muscular activities (mechanical stress, hormones, amino acids), there is a cascade of signals starting with protein kinase B, MAPK (54) (Figure 3B). The post-exercise feeding condition increases the phosphorylation of different signals leading to the stimulation of eukaryotic initiation of myofibrillar proteins. These acute effects contribute to positive changes in the 24-h muscle protein balance and quantitatively predict phenotypic adaptations with exercise training. However, what controls the upstream transcriptional changes remains to be defined.

Practical feeding recommendations for regular exercise practice

The information given here is directed at athletes or regular exercising individuals who should ingest adequate amounts of protein to maintain or increase their skeletal muscle mass status. A joint position of the American College of Sports Medicine, the American Dietetic Association and the Dietitians of Canada recommended protein intakes for endurance- and strength-trained athletes from approximately 1.2 to 1.7 g/kg per day (74). However, the scientific literature reveals a wide variety of practical conducts, which promote the adaptation of muscle mass through specific food applications: how much, with or without carbohydrates, what type of protein, how, when? We shall try to separate the wheat from the chaff.

How much protein is needed?

There is mounting evidence that the timing of ingestion and the protein source during recovery influence the extent of muscle hypertrophy. Minor differences in muscle protein turnover appear to exist between young men and women. An adequate protein balance is the result of an equation between the quantity of protein ingested per day and the amount of protein utilized under exercise conditions. As detailed previously, one could estimate this balance using the N intake by food and the N release in waste (mainly in urine). The NBal has been utilized for a long time (see Ref. 30), even though this is not considered to be the most accurate method. However, it represents an indirect method to evaluate the daily balance between protein intake using a daily food questionnaire and nitrogen release from protein degradation (mainly muscle mass) by urine collection. Table 5 gives an example of NBal recorded in young athletes (75, Poortmans JR, unpublished data).

Figure 4 shows the scattered distribution of NBal among young orienteering athletes and bodybuilders engaged in regular training. It appears that, under exercise conditions, the general adult population can easily equilibrate its NBal with a mean daily intake of 1.25 g protein/24 h. In one study on young gymnasts, using both NBal and the ^{15}N -glycine technique, we were able to observe a positive net protein

balance (+ 0.61 g protein/24 h) with a mean protein intake of over 1.39 g protein/24 h during a training season (76). Additional investigations on whole-body protein turnover (77) and skeletal muscle fractional synthetic rates in trained endurance humans (78) suggest that a protein intake of 1.2 g/24 h (or 10-12% of total energy) should achieve a positive NBal. A recent survey by Slater and Phillips (79) reported a protein intake that ranged from 1.1 to 3.3 g/24 h among adult male strength and power athletes during their training. However, as mentioned above, there is no evidence that even these strength athletes absorb more than 1.25 g protein/24 h.

How much protein is safe? A daily intake of 8-12% protein seems to be adequate and well balanced over the entire life span (80). But would excess protein and amino acid intake have detrimental effects on the human organism? Already in 1981, Waterlow and Jackson (18) stated that excess dietary protein is immediately oxidized. Probably most nephrologists and internal medicine practitioners should be concerned about excess protein intake. Consumption of high-protein diets by humans may have relevance to the occurrence of osteoporosis and hypercalciuria. We evaluated the consequences of excess protein intake on glomerular filtration rate (creatinine clearance), glomerular membrane permeability (albumin urine excretion) and calcium metabolism (calcium urine excretion rate) (75). Protein intake below a mean of 2.8 g protein/24 h does not impair renal function in well-trained athletes, as indicated by measures of renal function. But all excess protein intake results in a waste of money and a higher nitrogen excess (essentially urea) in the organism. Protein supplementation under exercise conditions should be addressed to stimulate net MPS, and more specifically, the optimal proportion of EAA (81,82).

With or without carbohydrate?

It has been reported that hyperinsulinemia stimulates MPS rates (83-85) and inhibits protein breakdown (86), leading to protein accretion. A post-exercise feeding strategy that provides 1.2 g carbohydrate·kg⁻¹·h⁻¹ seemed to improve muscle fractional synthetic rate (85), but another study concluded that carbohydrate does not augment exercise-induced protein accretion versus protein alone (87). The current literature remains equivocal in terms of post-exercise protein accretion, with or without carbohydrate addition. A recent predominant argument has been proposed to reach a conclusive statement (88). In summary, this statement means that athletes involved in regular training could add

Table 5. Nitrogen balance (NBal) of athletes recorded using a food questionnaire (over a 7-day survey) and urine nitrogen determination (mean of two 24-h collections over the week).

Athletes (N)	Gender	Age (years)	NBal (mean ± SEM) (g protein/24 h)	NBal > than 0* (g protein/24 h)
Running (5)	M	19-23	1.46 ± 0.07	1.22
Rowing (10)	M	15-20	1.28 ± 0.07	1.15
Cycling (12)	M	17-21	1.59 ± 0.09	1.37
Swimming (27)	M, F	11-18	1.52 ± 0.14	1.50
Gymnastics (13)	F	8-11	1.61 ± 0.36	1.39
Gymnastics (9)	F	15-16	1.12 ± 0.18	0.85
Basketball (14)	M	19-39	1.74 ± 0.13	1.18
Aerobic (16)	F	19-33	1.23 ± 0.05	1.17
Orienteering (15)	M	22-34	1.35 ± 0.12	1.30
Bodybuilding (19)	M	25-36	1.94 ± 0.13	1.30

Mean food protein intake (g/kg body weight) per 24 h were compared to total protein excretion (g/kg body weight) per 24 h to evaluate total nitrogen balance. The needed minimal protein intake per day was evaluated in each athlete group when NBal was equal to zero. *Positive NBal for all subjects investigated. Source: unpublished data from J.R. Poortmans. M = male; F = female.

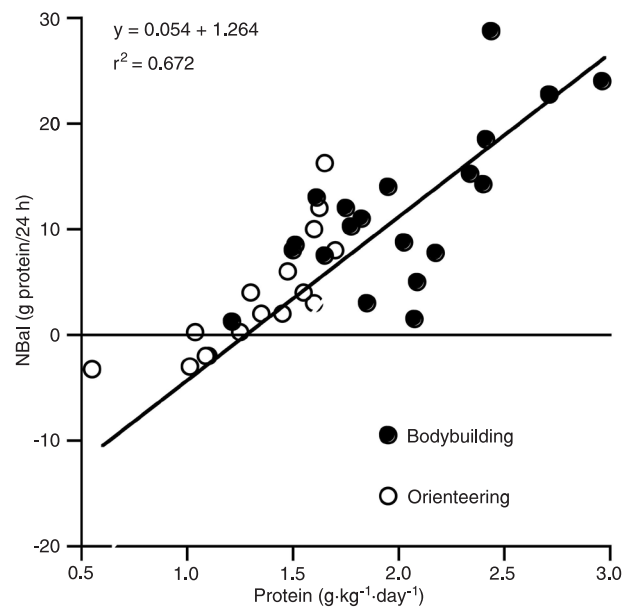


Figure 4. Distribution of nitrogen balance (NBal) among basketball players (filled dots) and orienteering runners (open dots) over 1-week survey. The zero line indicates an equal balance between nitrogen intake (by protein nutrition) and nitrogen excretion (by metabolic oxidations) over 24 h. Reproduced from Ref. 75, with permission.

some carbohydrate to their protein supplement since they have to keep a balanced diet both to replenish their glycogen store and to stimulate their muscle protein accretion.

What type of protein?

Animal or plant protein, all 20 amino acids, EAA, and

single leucine have been supplemented under resting conditions and mainly after exercise. Under resting conditions, Boirie et al. (89) demonstrated that dietary amino acid absorption is faster with whey protein than with casein, but there were no differential metabolic effects on skeletal muscle breakdown and synthesis when comparing feeding with casein or soy protein (90). As mentioned earlier, supplementation during exercise does not act on protein synthesis (91). But there is a total consensus that feeding during the recovery period from exercise induces muscle protein accretion. Let us remember, once more, that skeletal muscle represents about 40% of total mass and that nearly 80% of the three BCAA (leucine, isoleucine and valine) are located in muscle mass.

Quite reasonably, skimmed milk supplementation (± 18 g protein) has been proposed to athletes after resistance exercise (92-95). These publications showed greater lean mass accretion and functional performance induced by skimmed milk beverages. Whey proteins, or whey components, have been proposed as they contain nearly 50% of the EAA and about 26% of the BCAA. Moreover, whey proteins are obtained from skimmed milk after precipitation of casein, the latter being less digestible by humans. Different research teams used whey proteins to supplement athletes after resistance exercise to foster MPS (38,40,96-98). All these publications but one (38) concluded that an oral bolus of whey protein (10-48 g, or 0.3 g/kg lean body mass) taken immediately after resistance exercise stimulates MPS, and more specifically the myofibrillar protein fraction up to at least 6 h post-exercise (40). There is a general consensus indicating that whey protein increases and prolongs the mTORC1 signaling response. In fact, whey protein and its high leucine component stimulate the reversible phosphorylation of mTORC1 and 70-kDA ribosomal S6K1, thereby up-regulating the control mRNA binding to the 40S ribosomal subunit.

Why choose whey protein over other protein solutions (whole milk, EAA, soy, wheat, beef)? When compared to casein or whole milk, whey is more rapidly absorbed by the intestinal tract. Moreover, following resistance exercise, MPS is greater with whey protein than with casein or soy proteins. Already in 2003, Bohé et al. (21) demonstrated that human MPS is modulated by the extracellular (blood concentration) availability of EAA, and several publications have confirmed this observation following resistance exercise (39,84,99). However, these EAA beverages (with or without leucine enrichment) are more costly than whey protein products. Eventually, whey may also improve immune function and have gastrointestinal health benefits for physically active people.

And what about wheat protein from cereals, or beef proteins? Wheat contains moderate amounts of protein (8-12%) mainly composed of storage proteins (gluten, 80-85% of total wheat protein), with a deficiency of EAA (lysine, threonine). Moreover, wheat protein has a lower postprandial

nitrogen retention compared to animal protein. Haub et al. (51) analyzed the effect of beef protein versus soy protein sources on resistance-trained older men (65 ± 5 years) and concluded that muscle strength and size were not influenced by a predominant source of protein with adequate total protein intake (a mean of 1.1 g protein/24 h).

Would exercise be implicated in collagen MPS modifications? A study by Holm et al. (100) showed no effect of feeding (17% milk protein) on resting muscle collagen FSR (100). In addition, they realized that feeding after exercise also had no effect on intramuscular collagen synthesis. This suggests that the connective tissue collagen is differently regulated compared to the contractile structure in response to nutrients.

Concluding remarks and proposals

In 2001, Rennie (101) suggested that "muscle is acutely sensitive to amino acids, that exercise probably increases the anabolic effects of amino acids by a separate pathway, and that for this reason, it is unlikely that accustomed physical exercise increases protein requirements". This view was repeated in 2009 stating that "although not yet proven, it seems likely to us that any high-quality protein source, such as beef, egg, or soy, will be as good as milk for muscle protein accretion" (32). Moreover, the Institute of Medicine of the National Academies in its "Dietary Reference Intakes" concluded that "no additional dietary protein is suggested for healthy adults undertaking resistance or endurance exercise" (6). The numerous publications cited in the previous sections might be evidence contradicting these two proposals. Thus, let us be more convincing!

Indeed, the FAO/WHO estimates of adult protein requirements using nitrogen balance have evolved from 0.60 g·kg⁻¹·day⁻¹ in 1973 to 0.75 in 1985, and the 2005 requirements from the Institute of Medicine reached a value of 0.80 g·kg⁻¹·day⁻¹. However, those recommendations are focused on individuals with moderate- or medium-intensity physical activity. The many observations and suggestions from different research teams insist on a well-balanced diet for athletes that meets energy demands with varied sources of high-quality protein (102). The daily amount of protein should represent between 12 and 15% of the total energy requirement. As shown above, timing of ingestion, co-ingestion of nutrients and the type of protein may all influence protein accretion (103). Thus, a reasonably higher protein intake may be appropriate for some athletes (104) but we are convinced that an appropriate diet survey should be applied for several days, together with nitrogen-balance assays, to evaluate the real need for protein intake. It appears that a mean protein intake of 1.25 g·kg⁻¹·day⁻¹ is sufficient to compensate for the enhanced muscle protein degradation during prolonged exercise sessions (resistance and endurance types). As suggested by several publications, a bolus

of 15-20 g protein drink may be needed immediately after stopping exercise to stimulate muscle protein and tendon collagen turnover. Let us also keep in mind that any excess protein intake is useless.

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