Protein metabolism in exercising humans with special reference to protein supplementation

Anssi Manninen
Opinnäytetutkielma
Liikuntalääketieteen
koulutusohjelma
Kuopion ylipisto
Fysiologian laitos
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Abstract

A variety of studies using both humans and animals have shown that the rate of protein synthesis is generally depressed during exercise. After exercise, protein synthesis increases for periods up to 48 hours before declining to baseline values. Human muscle can oxidize at least seven amino acids. Of these amino acids, however, oxidation of only the branched chain amino acids (leucine, isoleucine, and valine) appears to be increased during catabolic states such as exercise.

Recent scientific studies indicate that for physically active individuals the recommended daily protein intake should be as high as 1.6-1.8 g · kg^{-1}. However, testimonials from athletes who believe that their success depends on consumption of large amounts of protein and energy suggest that additional research is necessary before the question of protein need in those attempting to increase lean mass is settled. Despite recommended protein requirements, special protein supplementation is unnecessary for most who consume a varied diet containing complete protein foods, assuming energy intake is sufficient to match the additional expenditures of training and competition.

There is evidence that ingestion of oral essential amino acids results in a change from net muscle protein degradation to net muscle protein synthesis after heavy resistance exercise in humans similar to that seen when the amino acids were infused. Moreover, there is some evidence that the response of net muscle protein synthesis to consumption of an oral essential amino-acid carbohydrate solution immediately before exercise is greater than when the solution is consumed after exercise.

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1 Introduction

1.1 Basic facts about proteins

Proteins consist of long chains of subunits called amino acids. As the name implies, each amino acid contains an amino group (NH_2) on one end of the molecule and a carboxylic acid group (COOH) on another end. There are approximately twenty different amino acids, each with a distinct structure and chemical properties that are used to build proteins (**Figure 1**).
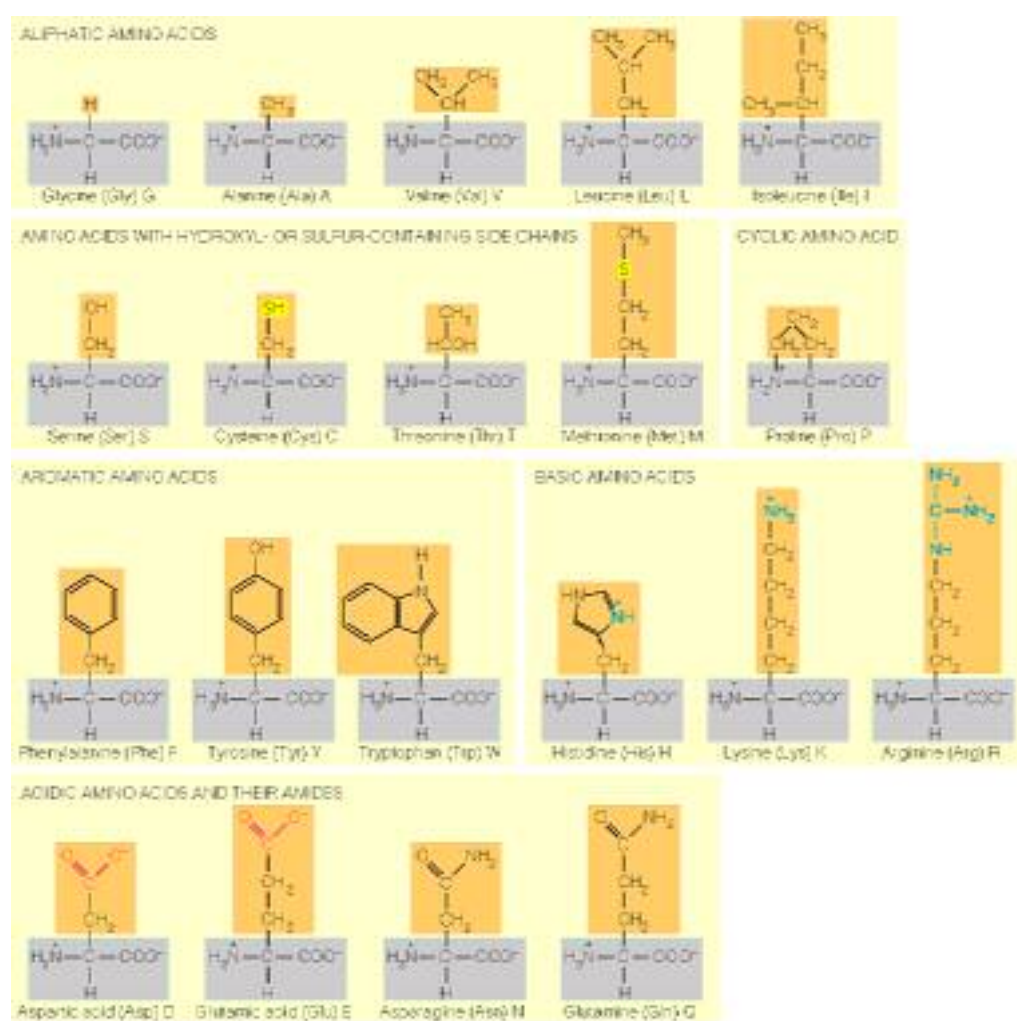
Figure 1. The Amino Acids Found in Proteins. Below each amino acid are its name, its three-letter abbreviation, and its one-letter abbreviation. *Reproduced from* Mathews et al. 2000.

When amino acids are joined together by dehydration synthesis, the hydrogen from the amino end of the one amino acid combines with the

hydroxyl group of carboxylic end of another amino acid. The bond between adjacent amino acids is called a peptide bond, and the compound formed is called a peptide (**Figure 2**). Two amino acids bound together is called a dipeptide; three, a tripeptide. When numerous amino acids are joined in this way, a chain of amino acids, or a polypeptide, is produced.
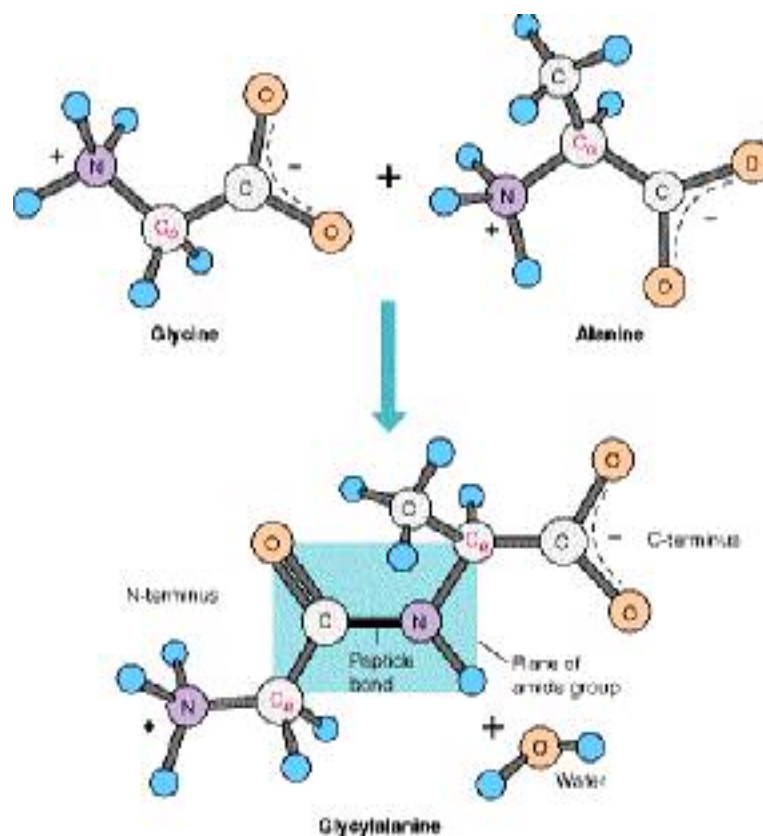
Figure 2. **Formation of a Peptide.** When two amino acids join, a peptide forms. Here the peptide glycylalanine (Gly-Ala) is depicted as being formed by removal of a water molecule when glycine is linked to alanine. *Reproduced from* Mathews et al. 2000.

The lengths of polypeptide chains vary widely. A hormone called thyrotropin-releasing hormone, for example, is only three amino acids long, whereas myosin, a muscle protein, contains approximately 4500 amino acids. When the length of a polypeptide chain becomes very long (containing more than approximately 100 amino acids), the molecule is called a protein.

Because of their tremendous structural diversity, protein can serve a wider variety of functions than any other type of molecule in the body. Many proteins, for example, contribute significantly to the structure of tissues and this way play a passive role in the functions of these tissues. Many proteins

play a more active role in the body, where specificity of structure and function is required. Enzymes and antibodies, for example, are proteins – no other type of molecule could provide the vast array of different structures needed for their tremendously varied functions.

1.2   Nitrogen balance

A nitrogen balance exists in organisms, when nitrogen intake (protein) equals nitrogen excretion as follows:

$$\text{Nitrogen balance} = N_t - N_u - N_f - N_s = 0$$

where N_t = total nitrogen intake from food; N_u = nitrogen in urine; N_f = nitrogen in feces; N_s = nitrogen in sweat.

If the body is in positive nitrogen balance, then protein is retained as new tissue is being synthesized. This is often observed in children, during pregnancy, in recovery from illness, and during resistance exercise training where protein synthesis occurs in muscle cells.

A greater output of nitrogen relative to its intake indicates protein use for energy and a possible encroachment on the body´s available amino acids, primarily those in skeletal muscle. If this occurs, a negative nitrogen balance can exist even at levels of protein intake above the standards established as the minimum requirements. This could happen if the body catabolizes protein because of lack of other energy nutrients.

For example, an individual who participates regularly in heavy training may consume adequate or excess protein but inadequate energy from carbohydrate or lipid. Consequently, protein becomes used as a primary energy fuel, the result being a negative protein (nitrogen) balance.

1.2.1 Nitrogen balance studies

Nitrogen balance is a laboratory technique by which both consumption and excretion of all nitrogen is meticulously quantified and the net difference calculated. On average, dietary protein is 16% nitrogen so protein retention or loss calculated by multiplying this net nitrogen difference by 6.25, i.e., 100/16.

The amount of protein necessary to elicit balance, i.e., when intake and excretion are exactly equal, can be determined (Y intercept in **Figure 3**) by measuring nitrogen excretion at a variety of different protein intakes. This quantity of protein is though to be the dietary requirement.
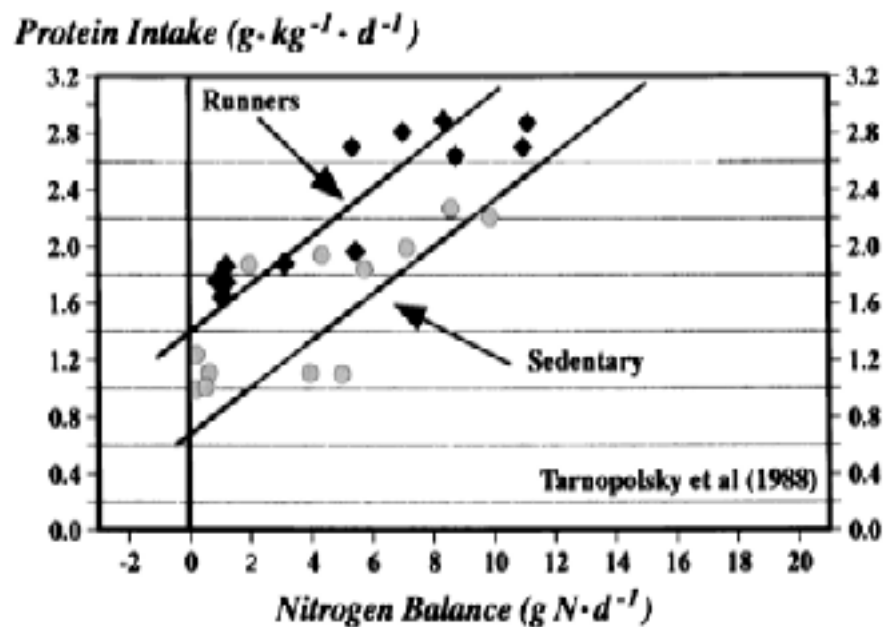
Figure 3. **Effect of Quantity of Dietary Protein on Nitrogen Balance in Sedentary Subjects Versus Runners.** Notice that nitrogen retention is linearly related to protein intake and that the runner´s data are above and the left of those of the sedentary subjects (Y intercept is greater in runners) indicating that the dietary protein requirement is increased by a regular running program. However, they were studied after a period of very high protein intake that followed one already twice the RDA, with no attempt at randomly ordering the treatment. Further, no low-protein diet was examined and no individuals ever experienced negative nitrogen balance. *Data from* Tarnopolsky et al. 1988.

However, the nitrogen balance method is recognized to have major limitations, the principal problems being

- The difficulty of identifying all the routes of nitrogen excretion

- The lack of adequate precision

- The assumption of linearity between nitrogen retention and protein intake.

- The problem of how long it takes to adapt at a given experimental intake

- The observed tendency to overestimate positive nitrogen balance

Another way to investigate the dietary protein requirements involves the use of metabolic tracers which make it possible to see into the "nitrogen status black box" by assessing which components of protein metabolism are affected by an exercise or dietary treatment. This is an improvement when compared with the nitrogen balance technique.

1.3   Metabolic tracers

1.3.1   Basic facts about metabolic tracers

Although the number of neutrons in the nucleus of an atom is often equal to the number of protons, many chemical elements can exist in multiple forms, called isotopes, which differ in the number of neutrons they contain. For example, the most abundant form of the carbon atom, ^{12}C, contains 6 protons and 6 neutrons, and thus has an atomic number of 6. Protons and neutrons are approximately equal in mass; therefore, ^{12}C has an atomic weight of 12. The radioactive carbon isotope ^{14}C contains 6 protons and 8 neutrons, giving it an atomic number of 6 but an atomic weight of 14.

A tracer is a molecule in which a normally occurring atom or atoms is substituted for by a less frequently occurring isotope of the same elemental atom or atoms. Carbon-13 (^{13}C) is a nonradioactive isotope of carbon that is naturally abundant in nature. Approximately 1% of all carbon is this heavier isotope, ^{13}C. This isotope is difficult to utilize and trace in metabolism, but, for the same reason, it is becoming the isotope of preference for human experimentations. The presence of carbon-13 is detected on the basis of its mass.

There are two basic strategies for using isotopes to study metabolism: *1)* pulse injection and *2)* continuous infusion. In the first approach, all of the isotope is injected into a rapidly mixing pool (e.g., the blood) in a single rapid (bolus) injection. In the second, the isotope is added at a continuous, set rate. A third approach is the primed continuous-infusion technique, which is combination of the other two.

1.3.2   Measuring of protein synthesis

Many of the early studies measuring protein synthesis involved laboratory animals. Protein synthesis rate was measured as the incorporation of a radioactively labeled amino acid into muscle protein over a specific time period, expressed, for example, as nanomoles of amino acid incorporated per gram of muscle tissues per hour.

Typically, phenylalanine is used for incorporation studies, as it is not metabolized by muscle, thus its only fate in muscle is to be used to make proteins (Houston 2001). The rate of protein synthesis in specific fractions of muscle proteins can be obtained by isolating mitochondria, contractile proteins, or soluble cytosolic proteins and determining the incorporation rate of labeled phenylalanine in each fraction per hour (Houston 2001).

In studies with humans, ethical considerations usually preclude the use of radioactive amino acids for non-medical studies. Therefore, scientists have employed isotopically labeled amino acids that are not radioactive, but which can be measured because of differences in their masses. These are known as stable isotopes.

The amino acid enriched with a particular stable isotope can be infused at a constant rate into a vein. We know that it will be used to make proteins. Therefore, muscle samples are obtained by biopsy at specific intervals and the amount of the amino acid with the stable isotope can be determined over time. Leucine, labeled at carbon 1 with a isotope of carbon ($1\text{-}^{13}C$-leucine) as opposed to normal isotope (^{12}C), is often used. Recent experiments have also used phenylalanine labeled with deuterium.

1.4   Regulation of protein synthesis

Although deoxyribonucleic acid (DNA) contains the information specifying the amino acid sequences in proteins, it does not itself participate directly in the assembly. Most of a cell´s DNA is in the nucleus, whereas most protein synthesis occurs in cytoplasm. The transfer of information from DNA to the site of protein synthesis is the function of ribonucleic acid (RNA) molecules, whose synthesis is governed by the information coded in DNA.

Genetic information flows from DNA to RNA and then to protein. The process of transferring genetic information from DNA to RNA in the nucleus is known as transcription (**Figure 4**); the process that uses the coded information in RNA to assemble a protein in the cytoplams is known as translation (**Figure 5**).

There are three different types of RNA, each of which plays an independent and entirely different role in the protein formation. They are

- Messenger RNA (mRNA), which carries the genetic code to the cytoplasm for controlling the formation of the proteins.

- Transfer RNA (tRNA), which transports activated amino acids to the ribosomes to be used in assembling the protein molecules.

- Ribosomal RNA (rRNA), which, along with approximately 75 different proteins, forms the ribosomes. The ribosomes are the physical and chemical structures on which protein molecules are actually assembled.
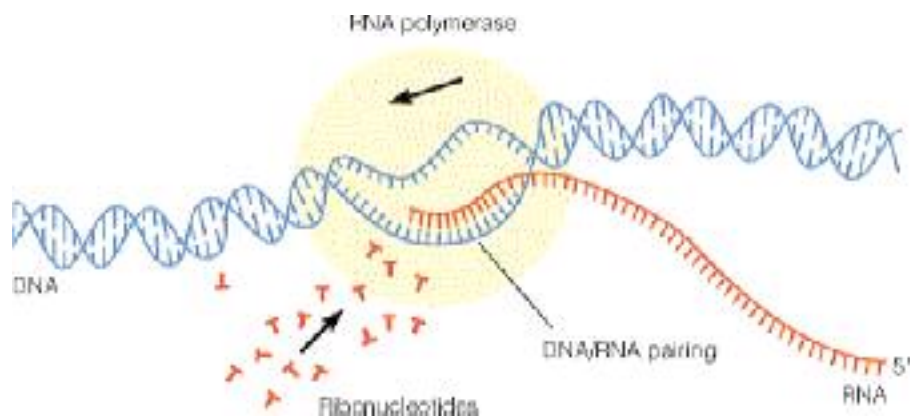
Figure 4. **The Basic Principles of Transcription.** An enzyme (RNA polymerase) travels along a DNA molecule, opening the double strand and making an RNA transcript by adding one ribonucleotide at the time. It copies the oligonucleotide sequence from only one of the two DNA strands. After the enzyme passes, the DNA rewinds. *Reproduced from* Mathews et al. 2000.
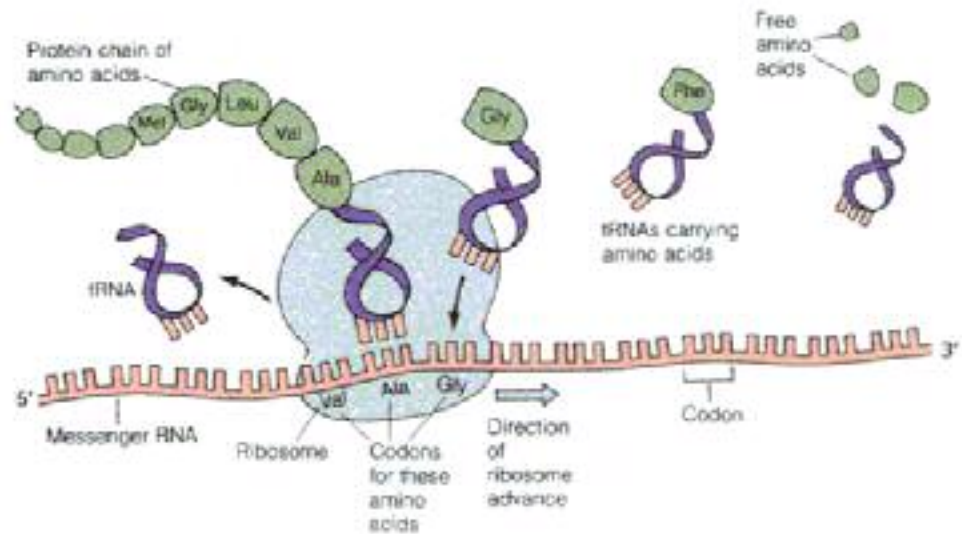
Figure 5. **The Basic Principles of Translation.** A messenger RNA molecule is bound to ribosome, and transfer RNA molecules bring amino acids to the ribosome one at the time. Each tRNA identifies the appropriate codon on the mRNA and adds this amino acid to the growing protein chain. The ribosome travels along the mRNA, so the genetic message can be read and translated into a protein. *Reproduced from* Mathews et al. 2000.

In order to have a net increase in protein synthesis so that there is an increase in the concentration of a protein in a cell, its rate of synthesis would have to increase or its breakdown decrease, or both. There are at least four ways in which the concentrations of protein in a cell could be changed:

- The rate of synthesis of the mRNA that codes for the particular protein(s) could be increased (know as transcriptional control).

- The rate of synthesis of the polypeptide chain by the ribosomal-mRNA complex could be increased (know as translational control).

- The rate of degradation of the mRNA could be decreased (also translational control).

- The rate of degradation of the protein could be decreased.

The table 1 outlines some of the conditions or factors affecting protein synthesis. Many of these conditions and factors are interrelated.

Table 1. Some Conditions and Factors Affecting Protein Synthesis

Conditions or factors	Effect on Rate of Protein Synthesis
Decreased protein intake	Decreased
Decreased energy intake	Decreased
Increased cellular hydration[a]	Increased
Decreased cellular hydration[a]	Decreased
Increased intake of leucine in presence of sufficiency of other amino acids	Increased
Increased intake of glutamine in presence of sufficiency other amino acids	Increased
Lack of nervous stimulation	Decreased
Muscle stretch, or exercise	Increased
Overtraining	Decreased
Testosterone (and anabolic steroids)	Increased
Growth hormone (GH)	Increased
Insulin-like growth factor 1 (IGF-1)	Increased
Normal thyroxine levels[b]	Increased
Excess thyroxine[b]	Decreased
Catecholamines[c] (including synthetic β-adrenergic agonist such as clenbuterol)	Increased
Glucocorticoids	Decreased
Physical trauma, infection	Decreased

Data from Di Pasquale 1997.

[a]It is important to understand that cellular hydration refers to an intracellular state and as such is different from extracellular hydration that is manifested either as water retention or volume depletion (extracellular dehydration) as measured by the degree of peripheral edema, overall blood volume, blood pressure, and serum concentrations of electrolytes.

[b]Thyroxine (T_4) and triiodothyronine (T_3) stimulate both protein synthesis and degradation – depending on their levels and the presence of and levels of other hormones and regulators. In physiological concentrations thyroid hormones stimulate the synthesis as well as the degradation of proteins, whereas in higher or supraphysiological doses protein catabolism predominates – as a part of the overall hypermetabolism that occurs. Thus, hypothyroidism suppresses muscle growth while hypothyroidism increases protein catabolism.

[c]Although increases in catecholamines can be generally considered catabolic, recent work has again pointed out the possible anabolic effects of these hormones. A recent study has shown that epinephrine directly inhibits proteolysis of skeletal muscle via the β-adrenoceptors (Kadowaki et al. 1996). However, the decrease in proteolysis is likely more than offset by a decrease in protein synthesis, resulting in an overall catabolic response.

1.5   Effects of exercise and diet on protein synthesis

A variety of studies using both humans and animals have shown that the rate of protein synthesis is generally depressed during exercise (Houston 2001). *After exercise, protein synthesis increases for periods up to 48 hours before declining to baseline values* (**Figure 6**). This has been shown following both endurance and resistance exercise, although the increase in protein synthesis with resistance exercise tends to be greater than what is observed following endurance exercise (Houston 2001).
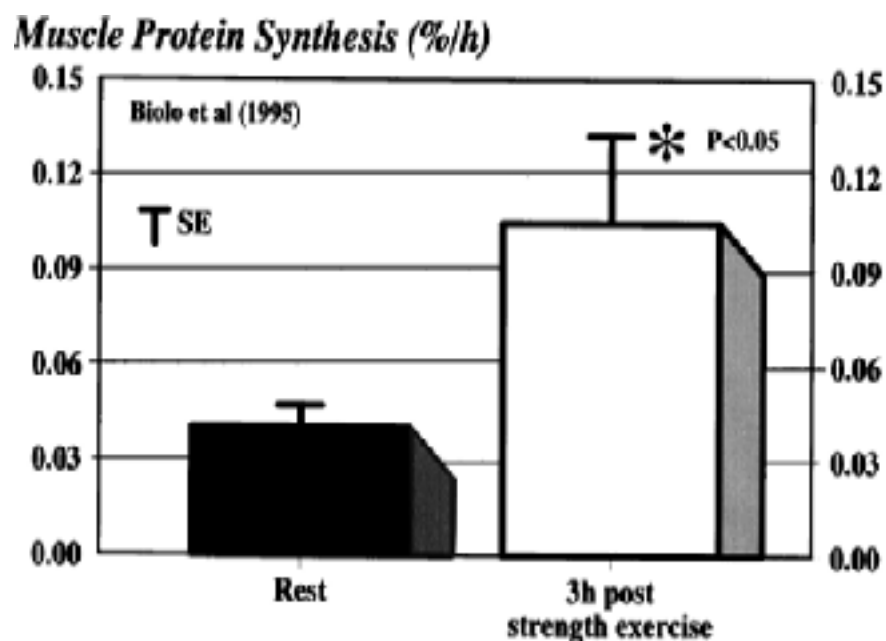
Figure 6. **Effect of a Strength Training Session on Muscle Protein Synthesis.** *Data from* Biolo et al. 1995.

In the fasted state, muscle protein synthesis is increased following resistance exercise (Houston 2001). This suggests that exercise by itself will stimulate synthesis. If the resistance or endurance training is followed by ingestion of carbohydrates or carbohydrates and protein, there is a dramatic improvement in muscle protein synthesis. This points to the important synergistic effect of food intake and exercise, particularly if food intake closely follows the end of exercise. One of the explanations for the effect of food is that the blood insulin concentration is elevated.

Recent studies also demonstrate that ingestion of a mixture of amino acids after resistance exercise stimulates muscle protein synthesis, without leading to an increase in insulin. These studies suggest that simply increasing blood amino acid concentrations can stimulate post-exercise muscle protein synthesis, likely by mass action effect.

How do exercise and diet increase protein synthesis so quickly following exercise? Houston (2001) suggests that they increase the rate of trancription of a number of genes, leading to a general increase in the content of a variety of mRNA molecules. Alternatively, the exercise and diet could increase the transcription of genes for rRNA or tRNA, leading to increased capacity of protein synthesis.

This explanation is highly unlikely given the short period of time in which the exercise or exercise and diet effect is noted – too short to transcribe genes, modify RNA, and get it into function form in the cytosol where protein synthesis takes place.

Moreover, these changes in protein synthesis take place without a measurable increase in total RNA or specific mRNA molecules. Therefore, the rate of protein synthesis increases without a change in total RNA, indicating an increased efficiency of translation (Houston 2001).

2 Role of protein in energy production

2.1 Basic facts about energy production

There are three basic ATP-synthesizing pathways in muscle. Two are anaerobic and one is aerobic. Phosphagen mobilization is the simplest mechanism for generating ATP. Creatine phosphate (CP), catalyzed by creatine phosphokinase, forms ATP from ADP:

$$CP + ADP + H^+ \leftrightarrow ATP + creatine$$
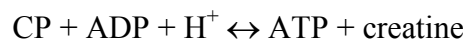
Anaeorobic glycolysis, the partial catabolism of glucose to lactate is the second anaerobic means of forming ATP:

$$Glucose + 2\ ADP \rightarrow 2\ lactate + ATP$$
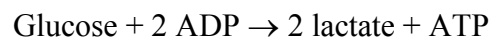
If glycogen is the substrate instead of glucose, and extra mole of ATP is generated. *Not only carbohydrates but amino acids can be anaerobically catabolized to form ATP*. Aspartate, for example, can be fermented to succinate or propionate. Unlike carbohydrates and protein, fats cannot be anaerobically reduced to form ATP.

Oxygen is required for the complete oxidation of substrates such as glucose, glycogen, fatty acids, and amino acids. The pathways by which such a complete oxidation are achieved are much more complex than the anaerobic pathways and involve the citric acid cycle (**Figure 7**).
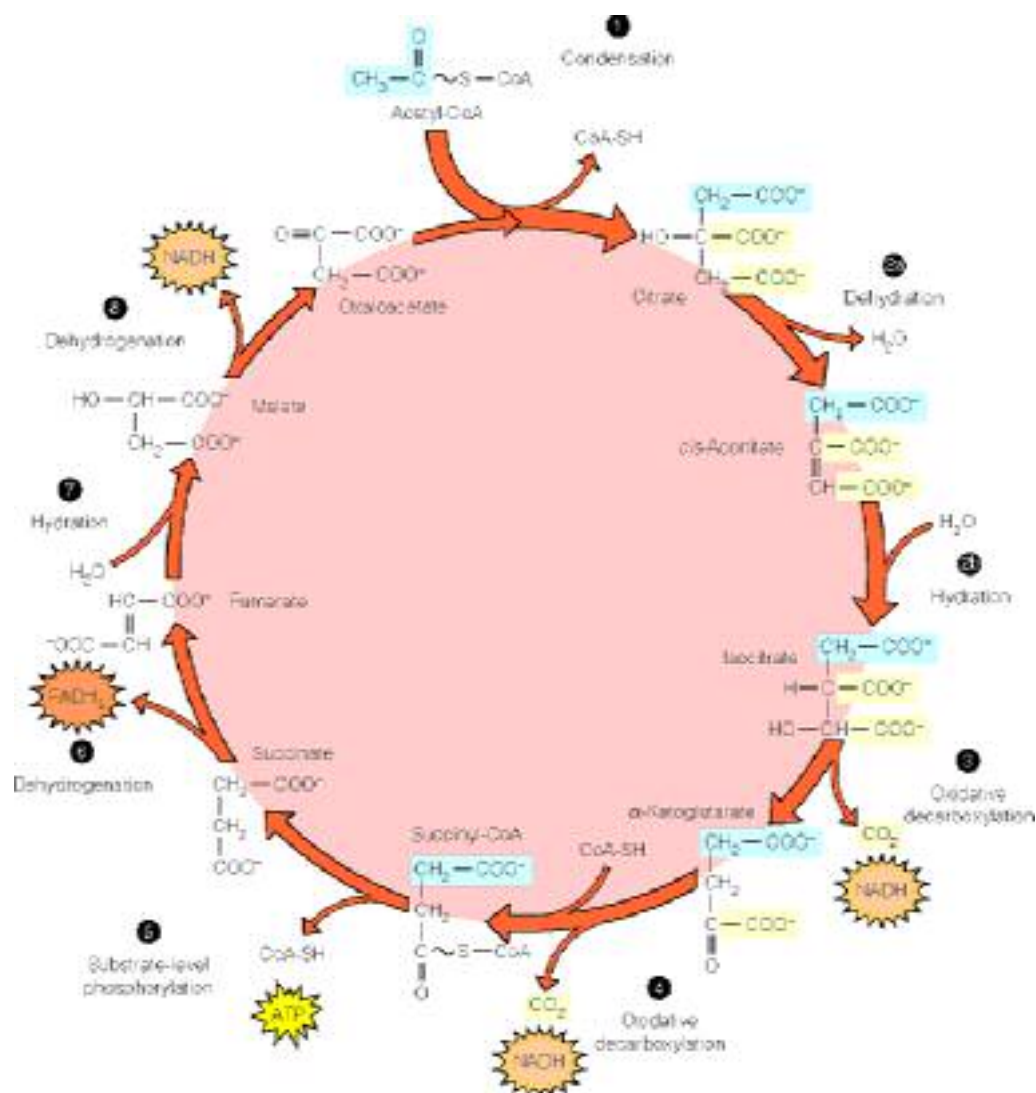
Figure 7. **The Citric Acid Cycle**. Entry into the citric acid cycle requires preparation of acetyl-CoA. Acetyl-CoA can be formed from the breakdown of either carbohydrates, fats, and proteins. *Reproduced from* Mathews et al. 2000.

Although the main function of dietary protein is its contribution to various anabolic processes, protein is also catabolized for energy. In well-nourished individuals at rest, the protein breakdown contributes between 2 to 5% of the body´s total energy requirements (McArdle et al. 2001).

Protein undergoes constant degradation because *1)* amino acids released during protein´s continual turnover that do not immediately participate to protein synthesis are catabolized for energy, *2)* dietary protein in excess of recommended values causes more amino acids to be converted to fat or catabolized to meet the body´s energy needs, and *3)* starvation, dieting,

prolonged exercise, and uncontrolled diabetes mellitus accelerate amino acid catabolism when carbohydrates are either unavailable or improperly used.

2.2 Deamination and transamination

During catabolism, protein must first be degraded into its amino acids components. Nitrogen is then stripped from the amino acid molecule in the process of deamination in the liver and is excreted from the body as urea (H_2NCONH_2). Deamination involves the removal of the amino group from the amino acid molecule.

A new amino acid can then be synthesized from the deaminated amino acid, or the remaining deaminated carbon compound can be synthesized into a carbohydrate or lipid, or it can be metabolized directly for energy. The urea formed in deamination (including some ammonia) leaves the body in the solution as urine.

In muscle, enzymes are available that facilitate nitrogen removal from certain amino acids and subsequently pass this nitrogen to other compounds in the biochemical reactions of transamination. This process involves the shifting of an amino group from a donor amino acid to an acceptor acid (keto acid), the acceptor thus becoming a new amino acid (**Figure 8**).
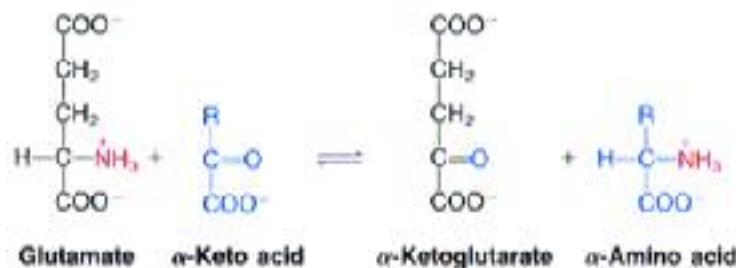
Figure 8. Transamination. Transamination reactions are catalyzed by enzymes called transaminases, or more properly, aminotransferases. Transamination involves transfer of the amino group, usually of glutamate, to an α-keto acid, with formation of the corresponding amino acid plus the α-keto derivative of glutamate, which is α-ketoglutarate. Like amino acid dehydrogenases, transaminases are enzymes that can function in either direction depending on the circumstances. A very common and important transaminase is glutamate-pyruvate transaminase (GPT). This is a major route by which alanine is utilized: pyruvate + glutamate ↔ alanine + α-ketoglutarate. A second common transaminase is glutamate-oxaloacetate transaminase (GOT): oxaloacetate + glutamate ↔ aspartate + α-ketoglutarate. This reaction yields the Krebs cycle intermediate (α-ketoglutarate) and amino acid aspartate. Passage through aspartate is the major route by which most nitrogen is excreted from the body. Most transaminase reactions, such as the GPT reaction, are freely reversible, and function depending on substrate availability. For example, pyruvate and glutamate have high concentrations in muscle, so the enzyme tends to operate left to right, with the formation and release of alanine (**Figure 13**). Conversely, GPT operates from right to left in the liver, using alanine to form pyruvate, which can be made into glucose (**Figure 13**). *Reproduced from* Mathews et al. 2000.

In both deamination and transamination, the resulting carbon skeleton of the non-nitrogenous amino acid residue can then be further degraded during energy metabolism.

2.3 Fate of amino acid after nitrogen removal

After deamination, the remaining carbon skeletons of α-keto acids such as pyruvate, oxaloacetate, or α-ketoglurate follows diverse biochemical routes, including following:

- *Gluconeogenesis*: 18 of the 20 amino acids serve as a source for glucose synthesis (**Figure 9**).

- *Energy source*: The carbon skeletons oxidize for energy because they form intermediates in citric acid cycle metabolism or related molecules (**Figure 10**).
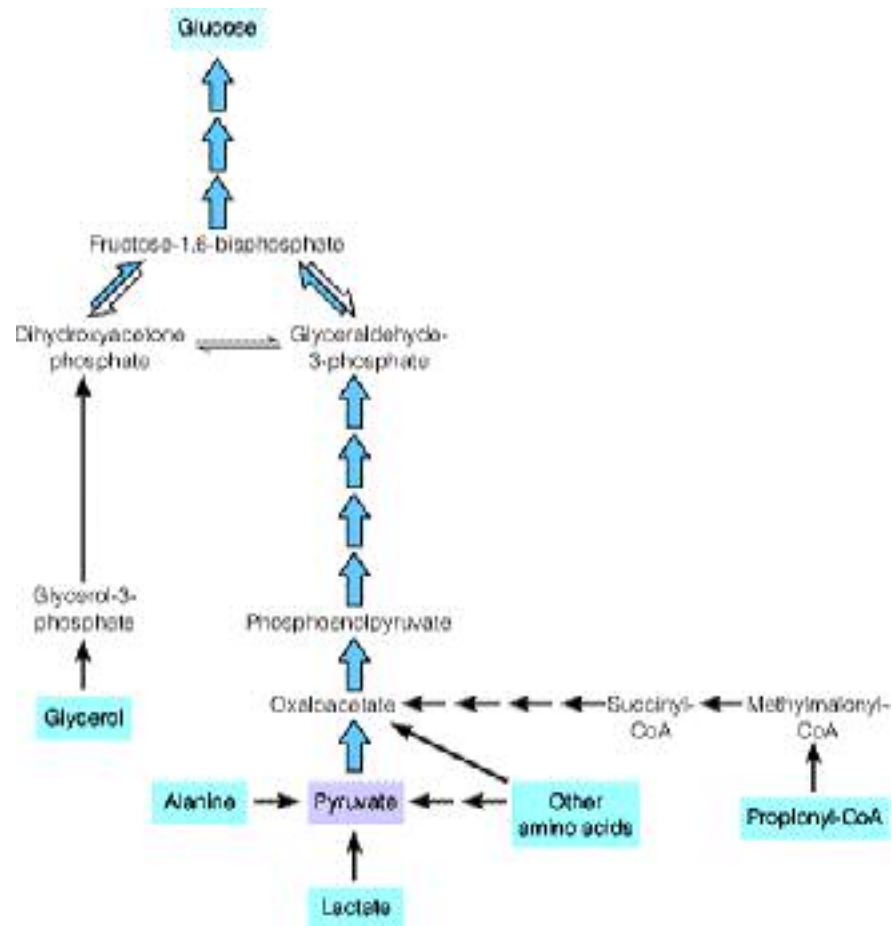
Figure 9. Outline of Pathways for Glucose Synthesis From the Major Gluconeogenic Precursors. Gluconeogenesis is defined as the biosynthesis of carbohydrate from three-carbon and four-carbon precursors, generally noncarbohydrate in nature. The primary gluconeogenic organ is the liver, with kidney cortex contributing in a lesser but still significant way. The major fates of glucose formed by gluconeogenesis are catabolism by nervous tissue and utilized by skeletal muscle. Many amino acids can readily be converted to glucose, primarily through degradative pathways that generate citric acid cycle intermediates, which can be converted to oxaloacetate. Such amino acids are called glucogenic (that is, able to be converted to glucose), although gluconeogenic is probably more accurate term. Among the 20 amino acids found in proteins, only the catabolic pathways for leucine and lysine do not generate glucogenic precursors. Among the hormones responsible for a rise in gluconeogenesis one must take into account adrenaline, glucagon, glucocorticoids, growth hormone, and insulin. These hormones can act either directly on the gluconeogenic enzymes or on the mobilization of precursors necessary for gluconeogenesis. On a moment-to-moment basis, however, these processes are controlled mainly by the glucocorticoids, insulin, and glucagon, whose secretions are reciprocally influenced by the plasma glucose concentration. The glucocorticoids increase the activity of the glucose-alanine cycle (**Figure 13**). Insulin decreases the supply of gluconeogenic substrates and inhibits the glucose-alanine cycle. Thus, *the exercise-induced hypoinsulinemia is a promoting factor in gluconeogenesis*. Under stressful conditions (e.g., hypoglycemia, trauma, vigorous exercise), increased secretion of other hormones such as adrenaline, cortisol, and growth hormone, and increased activity of the sympathetic nervous system, come into play. Their actions to increase hepatic glucose output and to suppress tissue glucose uptake are partly mediated by increases in tissue fatty acid oxidation. *Reproduced from* Mathews et al. 2000.
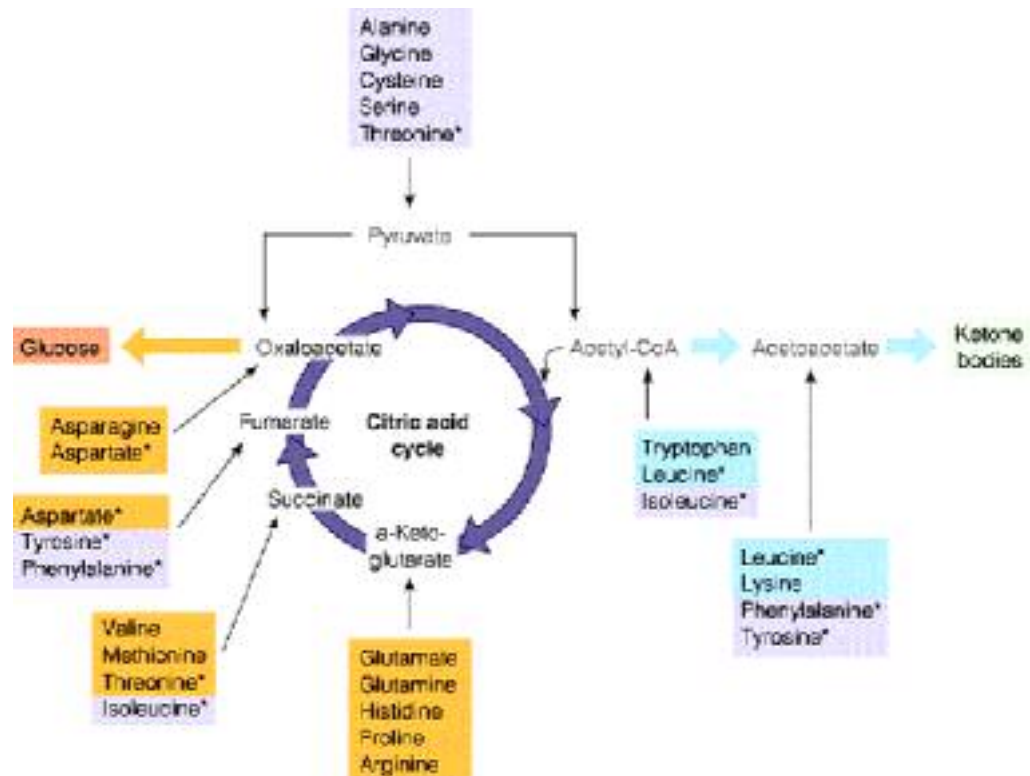
Figure 10. Fates of the Amino Acid Carbon Skeletons. Upon removal of their amine group, all amino acids form reactive citric acid cycle intermediates or related compounds. Amino acids with more than one route to entry to central pathways are marked by an asterisk. The carbon skeletons of ten amino acids yield acetyl-CoA, which enters the citric acid cycle directly. Five of the ten are degraded to acetyl-CoA via pyruvate. The other five are converted into acetyl-CoA and/or acetoacetyl-CoA, which is then cleaved to form acetyl-CoA. The five amino acids entering via pyruvate are alanine, glycine, serine, cysteine, and tryptophan. In some organisms threonine is also degraded to form acetyl-CoA; however, in humans it is degraded to succinyl-CoA. Alanine yields pyruvate directly on transamination with α-ketoglutarate, and the side chain of tryptophan is cleaved to yield alanine and thus pyruvate. Cysteine is converted to pyruvate in two steps, one to remove the sulfur atom, the other a transamination. Serine is converted to pyruvate by serine dehydratase. Both the β-hydroxyl and the α-amino groups of serine are removed in this single PLP-dependent reaction. Glycine has two pathways. It can be converted to into serine by enzymatic addition of a hydroxymethyl group. The second pathway for glycine, which predominates in animals, involves its oxidative cleavage into CO_2, NH_4^+, and a methylene group. Portions of the carbon skeleton of six amino acids – tryptophan, lysine, phenylalanine, tyrosine, leucine (**Figure 11**), and isoleucine (**Figure 11**) – yield acetyl-CoA and/or acetoacetyl-CoA; the latter is then converted into acetyl-CoA. *Reproduced from* Mathews et al. 2000.

The daily protein intake is used to maintain existing tissue protein, hormones, and enzymes. ***If more is taken in than needed, the "extra" is oxidized for metabolic needs, and fat mass is not increased*** (Powers and Howley 2001).

2.4 Role of protein in energy production during exercise

Over the past 100 years, the contention that protein is used only to limited extent as an energy fuel has generally been based on two observations: *1)* a protein´s primary role is to provide the amino-acid building blocks for tissues synthesis, and *2)* the finding of early studies that there was only minimal protein breakdown during endurance exercise as reflected by urinary nitrogen in the immediate 24-hour recovery period.

Recent research on protein balance in exercise, however, presents a compelling argument that protein is used as an energy fuel to a much greater extent than previously though, and that such protein utilization varies with energy expenditure and nutritional status.

There are three principal sources of amino acids for energy metabolism: *1)* dietary protein, *2)* plasma and tissue free amino acid pools, and *3)* endogenous tissue protein. All three sources are in equilibrium. Dietary protein is a relatively minor source of amino acids during normal exercise since ingesting a large protein meal prior to exercise is rarely done. The plasma pool of free amino acids is much smaller than the free amino acid pool of human skeletal muscle largely because skeletal muscle makes up approximately 40% of body weight and contains approximately 75% of the whole-body free amino acids (Di Pasquale 1997).

The free amino acid pools, however, are much smaller than the amounts of amino acids available from endogenous protein breakdown. It has been estimated that intramuscular amino acid pool contains less than 1% of the metabolically active amino acids (Di Pasquale 1997). It has also been estimated that the amount of leucine oxidized during a prolonged exercise bouts is approximately 25 times greater than the free leucine concentration on muscle, liver, and plasma (Di Pasquale 1997). Therefore, *the free amino acid pool is only a minor source of amino acids during exercise, whereas the most important source is endogenous protein breakdown.*

Human muscle can oxidize at least seven amino acids: leucine, isoleucine, valine, glutamate, asparagine, aspartate and alanine. Of these amino acids, however, oxidation of only the branched chain amino acids (leucine, isoleucine and valine, BCAA) appears to be increased during catabolic states such as exercise (Phillips 2002).

2.5   Oxidation of branched-chain amino acids (BCAA)

The branched-chain amino acids (leucine, isoleucine, and valine, BCAA) are so named, because they have a carbon chain which deviates or branches from the main linear carbon backbone. They are unusual in that they are catabolized mainly in skeletal muscle (Brooks et al. 2000) – where carbon skeletons provide an oxidizable source of substrate and where nitrogen residues participate in alanine formation.

The increased BCAA oxidation during exercise is due to the synergistic effects of a high abundance of BCAA in skeletal muscle protein (~ 20% of all muscle protein, by content) along with the fact that the activity of the enzymes responsible for the transamination and subsequent oxidation are also relatively high in muscle (Phillips 2002).

Degradation of leucine, isoleucine, and valine in humans starts with transamination followed by oxidative decarboxylation of the respective amino acids (**Figure 11**).

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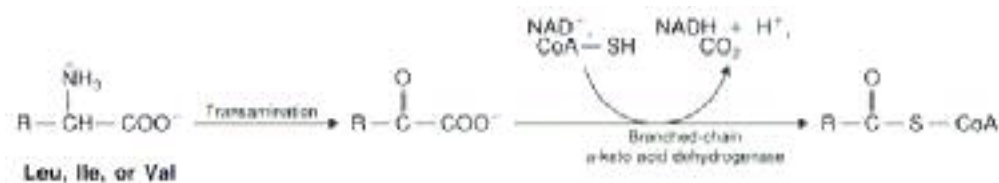
Figure 11. Degradation of Branched-Chain Amino Acids. The metabolism of BCAA begins with a transamination and the formation of glutamate and a α-keto acid. The glutamate so formed can then donate nitrogen to pyruvate and form alanine. The second step in leucine catabolism is the dehydrogenase step, which is also a decarboxylase. The remaining carbon atoms of leucine are then converted either to acetyl-CoA or to acetoacetate. The fate of these products is oxidation. *Leucine is therefore purely ketogenic.* The catabolism of the other BCAA proceeds somewhat differently. Isoleucine forms both acetyl-CoA and succinyl-CoA and is therefore both ketogenic and glucogenic. Valine produces succinyl-CoA and is glucogenic. According to work by Kasperek (1989), the dehydrogenase step is rate-limiting in the catabolism of BCAA during exercise and recovery. *Reproduced from* Mathews et al. 2000.

The latter reaction is carried out by a multienzyme complex, called the branched-chain α-keto acid dehydrogenase complex (BCKAD), which is similar in structure and mechanism to pyryvate dehydrogenase and α-ketoglutarate dehydrogenase complexes.

Exercise stimulates activation of BCKAD, due possibly to a decrease in the ratio of ATP/ADP, or in an increase in intramuscular acidity (May et al. 1987). In theory, however, because training induces an increase in the number of mitochondria, the maximal activity of BCKAD should also increase.

However, endurance training also results in the ability to better defend against changes in the ATP/ADP ratio (Phillips et al. 1996), hence, the proportion of the active form of BCKAD would be expected to decrease as a result of exercise. However, using leucine oxidation as a surrogate marker of BCKAD activity, the effect of training on this enzyme complex is entirely inconclusive with studies showing *1)* increases in leucine oxidation following training, *2)* no change and even *3)* decreases (Phillips 2002).

Because it appears that muscle does not take up BCAA during exercise, at least not in appreciable quantities (MacLean et al. 1994), the increase in leucine oxidation observed during exercise should arise, for the most part, due to an increase in muscle protein breakdown within the muscle. An increase in muscle proteolysis during exercise would then supply leucine and other BCAA

to the intramuscular free amino acid pool. In fact, MacLean et al. (1994) have shown that ingestion of BCAA reduces proteolysis, at least as measured by amino acid release across the exercising leg.

It is now apparent that as exercise progresses there is an increase in the concentration of plasma urea. This increase is coupled with a dramatic rise in nitrogen excretion in sweat, often without any change in urinary-nitrogen excretion (McArdle et al. 2001).

These observations account for earlier conclusions of minimal protein breakdown during endurance exercise because the early studies only measured nitrogen in urine. **Figure 12** illustrates that the sweat mechanism is an important means for excreting the nitrogen from protein breakdown during exercise.
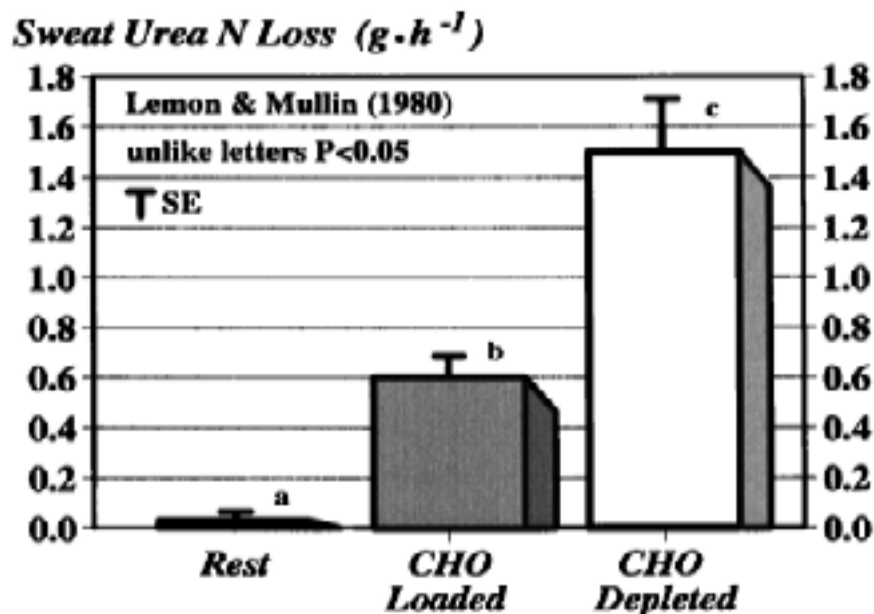
Figure 12. Excretion of Urea in Sweat at Rest, During Exercise After Carbohydrate Loading (CHO Loaded) and Carbohydrate Depletion (CHO Depleted).. *Data from* Lemon and Mullin 1980.

This study demonstrated a dramatic increase in sweat urea nitrogen in men previously depleted of glycogen stores and exercised for 1 h at 61% VO_{2max}, but no change in urinary urea nitrogen. The authors found that amino acid catabolism provided 10.4% of total energy for the exercise in CHO depleted

individuals while in the CHO loaded group protein provided 4.4% of energy needs.

The evidence that *1)* glucose inhibits leucine oxidation (Davies et al. 1982), *2)* urea excretion in urine and sweat is accelerated in glycogen-depleted subjects (**Figure 12**), and *3)* the BCKAD is activated to a greater extent in glycogen-depleted rats (Kasperek and Snider 1987; Wagenmakers et al. 1984) are all consistent with the idea that *there is an inverse relationship between the extent of BCAA oxidation and glycogen availability.*

Direct testing of this hypothesis was made by studying the responses of BCKAD complex and muscle and blood metabolites during exercise in trained subjects after either glycogen depletion or carbohydrate loading. It supports the idea that exercise in the glycogen-depleted condition is associated with more rapid increase in ammonia and lower increases in alanine, glutamate, and glutamine together with a fourfold increase in the activation of the enzyme complex (Wagenmakers et al. 1991). When subjects were CHO-loaded, no activation occurred.

The rise in leucine oxidation observed during exercise is exactly what would be expected from observations of an increased state of activity of the BCKAD in animal and human muscle. Because the Michaelis-Mentent constant (K_m) for the branched chain transaminase is high and the K_m for the L-system transporter is also high (Hundal et al. 1989), leucine oxidation should mainly depend on leucine delivery.

Fasting causes increases in plasma leucine concentration (as a result of whole-body protein breakdown), and starvation is associated with a fall in insulin and an activation of the BCKAD in muscle (Rennie et al. 1994). This helps to explain, why exercise in the fasted state causes a bigger increase in leucine oxidation than in the fed state.

Wolfe et al. (1984, 1987) claim that exercise is not associated with increases in urea production measured from urine or blood changes, an application of isotopic methods to urea turnover shows no increase in urea production in exercise.

Although artifacts in $^{13}CO_2$ production cannot explain the substantial rise in $^{13}CO_2$ production from ^{13}C leucine (which are matched by similar changes with ^{14}C-labeled leucine), there remains the difficulty of why increased BCAA oxidation does not show up in terms of urea production.

According to Rennie et al. (1994), possible difficulties are that the isotopic urea turnover values were methodologically flawed, that the work rates examined by Wolfe et al. were too low, or that changes are difficult to see because of the very large urea pool.

In chronically catheterized dogs exercise is clearly associated with increased hepatic ureagenesis (i.e., elevated portovenous urea differences) that hardly show up in the blood urea pool (Wasserman et al. 1991).

2.6 Interorgan exchange of amino acids during exercise

Many of the alterations in the hormonal ensemble during exercise favor protein and amino acid mobilization. As a result of exercise, there is an interorgan exchange of amino acids, particularly the BCAA, alanine, and glutamine. The main features of this interorgan exchange include (Di Pasquale 1997):

- The movement of the BCAA from the splanchnic bed (liver and gut) to skeletal muscle.

- The movement of alanine from muscle to the liver.

- The movement of glutamine from muscle to the gut.

The interorgan exchange of these amino acids has several functions including (Di Pasquale 1997):

- Maintaining amino acid precursors for protein synthesis.

- Assisting in the elimination of nitrogen wastes.

- Providing substrates for gluconeogenesis.

- Providing glutamine for gut and immune system function.

- Maintaining the purine nucleotide cycle.

2.7   The glucose-alanine cycle

One mechanism by which blood glucose homeostasis is maintained is the glucose-alanine cycle (**Figure 13**). During fasting, proteins are degraded to provide 100 g of glucose per day, which is used almost exclusively by the brain, nerves, and kidneys (Brooks et al. 2000).

Amino acids from degraded muscle proteins circulate to the liver, where deamination, and gluconeogenesis take place. Of the amino acids reaching the liver, alanine is by far the most important; half or more of the amino acids taken up by the liver are in the form of alanine. During fasting and starvation, the arterial level of alanine largely determines the rate of gluconeogenesis (Brooks et al. 2000).
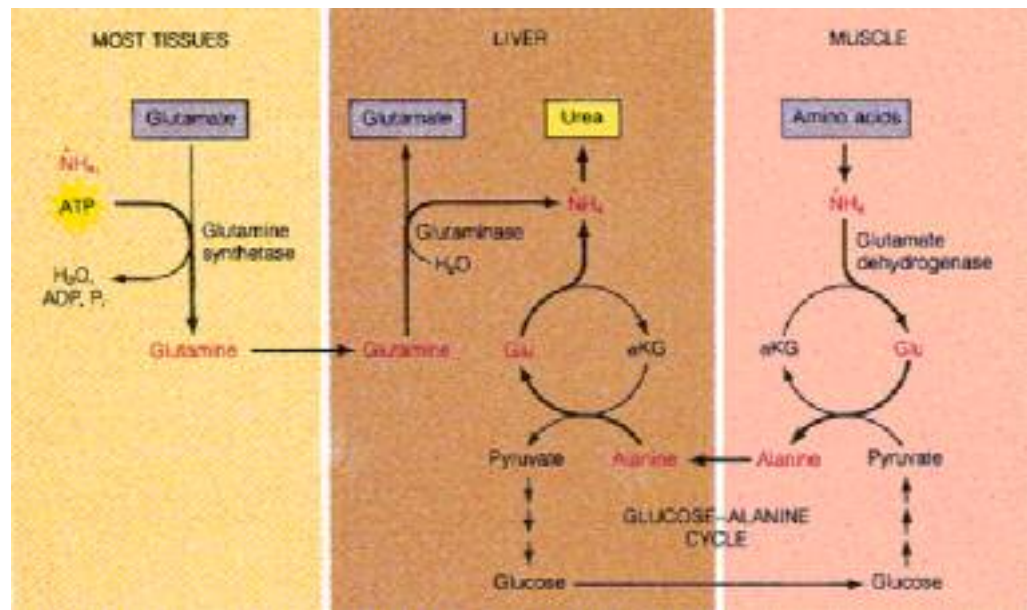
Figure 13. **Transport of Ammonia to Liver for Urea Synthesis.** Two mechanisms are involved in transporting ammonia from other tissues to liver for its eventual conversion to urea. Most tissues use glutamine synthetase to convert ammonia to nontoxic; and electrically neutral, glutamine. The glutamine is transported in the blood to the liver, where it is cleaved hydrolytically by glutaminase: glutamine + H_2O $\rightarrow$ glutamate + ammonia. Muscle, which derives most of its energy from glycolysis, uses a different route, the glucose-alanine cycle. Glycolysis generates pyruvate, which undergoes transamination with glutamate to give alanine and α-ketoglutarate. The glutamate in turn has acquired its nitrogen from ammonia, via glutamate dehydrogenase. The resultant alanine is transported to the liver, where it loses its nitrogen by a reversal of previous processes. This reversal yields ammonia for urea synthesis, plus pyruvate. The pyruvate undergoes gluconeogenesis to give glucose, which is released to the blood for transport back to the muscle or for nourishment of the brain. *This cyclic process helps muscle get rid of ammonia, with the carbon from pyruvate being returned to the liver for gluconeogenesis*. It has long been known that muscle liberates ammonia (NH_4^+) when it contracts. If creatine phosphate stores are maintained by intermediary metabolism in contracting muscle, then the ammonia must be provided by a source other than creatine phosphate. That source is thought to be the *purine nucleotide cycle*, as described by Lowenstein (1972). The purine nucleotide cycle is very active in contracting muscle; the cycle produces inosine monophopshate (IMP) from AMP, a citric acid cycle intermediate fumarate, and ammonia. The AMP is potentially useful in regulating metabolism, and the fumarate provides material that will eventually form oxaloacetate and combine with acetyl-CoA in operation of the citric acid cycle. Ammonia is one of the stimulators of a key glycolytic enzyme (phopshofructokinase), but the accumulation of ammonia can be toxic to the tissue. This accumulation is minimized by the formation of glutamate and glutamine. Studies by Meyr and Terjung (1980) indicate that PNC activity is particularly high in fast skeletal muscle during exercise. *Reproduced from* Mathews et al. 2000.

The alanine formed in muscle and released into the circulation most likely does not represent the catabolism of a protein rich in alanine content (a polyalanine). Rather, the alanine is newly (*de novo*) synthesized in muscle (Brooks et al. 2000).

Because the glucose-alanine cycle contributes to glucose homeostasis during fasting and starvation, it was logical for researchers to hypothesize that the cycle also operates during prolonged exhaustive exercise. This hypothesis was put forth by Felig and associates (1971), including Wahren and Ahlborg (1973).

Their results, based on quantitative measurements of muscle alanine release, liver uptake, and liver glucose release, indicate greatly elevated glucose-alanine cycle activity during exercise as compared to rest. According to their data, the glucose-alanine cycle may provide 5% of total fuels used during exercise.

Because several amino acids contribute to maintaining glucose homeostasis and substrate supply during exercise, perhaps the total contribution to the fuel supply by all amino acids is closer to 10 than 5% (Brooks et al. 2000).

2.8   The glucose-glutamine cycle

Results of recent experiments have shown that a glucose-glutamine cycle exists in the body as a major means for transporting carbon and nitrogen atoms from skeletal muscle to the kidneys for nitrogen (urea) excretion and gluconeogenesis.

With important differences, this cycle is similar to the glucose-alanine cycle. The differences are that in the glucose-glutamine cycle, glutamine (not alanine) is the transport mechanism and the kidneys (not the liver) are the sites of transamination and gluconeogenesis (Brooks et al. 2000).

In resting postabsorptive individual, the two glucose-amino acid cycles provide similar rates of gluconeogenesis, but unfortunately the quantitative role of glutamine as a gluconeogenic precursor in exercising humans has not yet been determined.

Because far more quantities of lactate are produced during exercise than alanine or glutamine, it is likely that the Cori cycle (with lactate as the gluconeogenic precursor) (**Figure 14**) will prove to be the more important mechanism for maintenance of glycemia during exercise than either the glucose-alanine or glucose-glutamine cycles (Brooks et al. 2000).
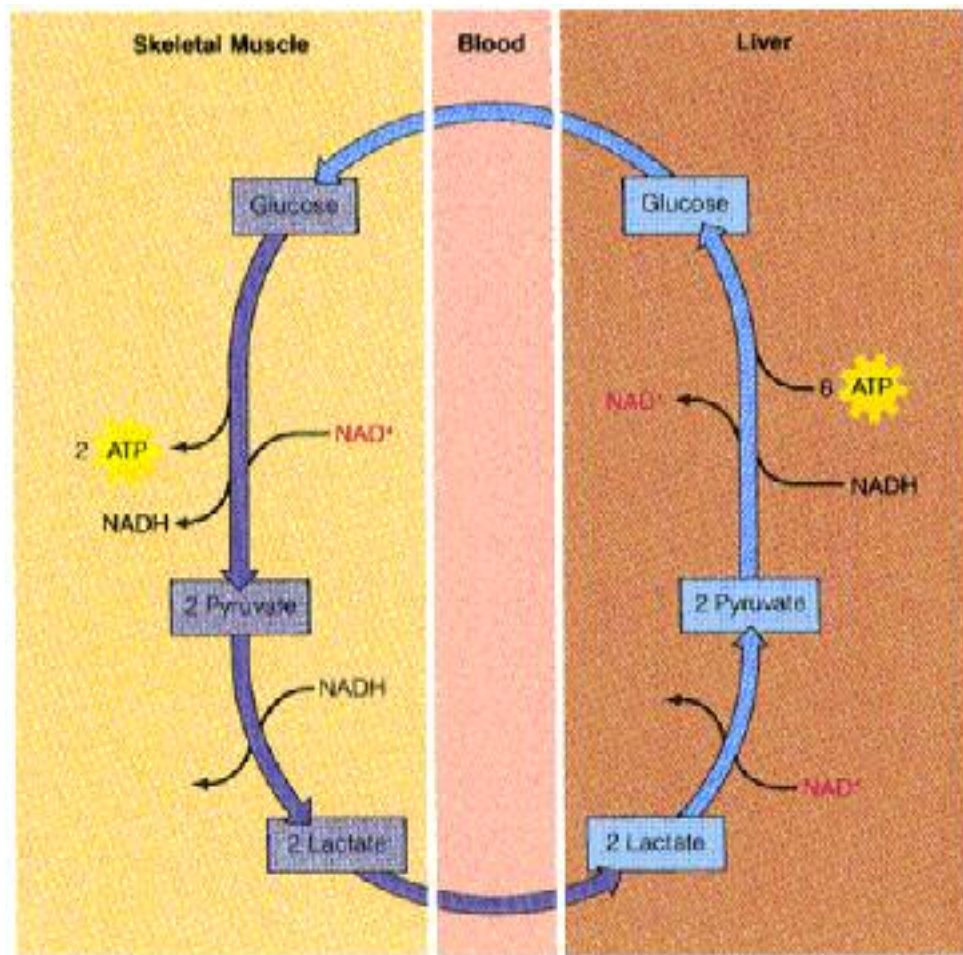
Figure 14. **The Cori Cycle.** Carl and Gerti Cori were among the first to recognize that the lactate and pyruvate produced by skeletal muscle could circulate to the liver and be made into glucose. The glucose so produced could then recirculate to muscle. Rapid glycolysis in skeletal muscle inevitably results in lactate production because of the activity and equilibrium constant (K_{eq}) of lactate dehydrogenase, and gluconeogenesis is an efficient way to reutilize the products of glycolysis, thereby providing for the maintenance of blood glucose and prolonged muscle glycolysis. *Reproduced from* Mathews et al. 2000.

3 Protein requirements of athletes

3.1 Basic facts about protein requirements

The term protein requirement means the amount of protein which must be consumed to provide the amino acids for the synthesis of those body proteins irreversibly catabolized in the course of the body´s metabolism. The requirement of dietary protein consist of two components:

- The requirement for the nutritionally essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) under all conditions, and for conditionally essential amino acids (arginine, cysteine, glutamine, glycine, histidine, proline, taurine, and tyrosine) under specific physiological and pathological conditions (for review see Di Pasquale 1997).

- The requirement for nonspecific nitrogen for the synthesis of the nutritionally dispensable amino acids (aspartic acid, asparagine, glutamic acid, alanine, and serine) and other physiologically important nitrogen-containing compounds such as nucleic acids, creatine, and porphyrins.

The recommended allowances for protein (nitrogen) are based upon experiments in which normal requirement is defined as the intake necessary to achieve zero balance between intake and vs. output.

According to US Food and Nutrition Board (1989), any increased need for protein induced by exercise does not exceed the safety buffer included in the current RDA ($0,8$ g protein $\cdot$ kg^{-1} $\cdot$ day^{-1}). However, it is important to realize that this opinion is somewhat speculative as the recommendation is based on experiments completed on subjects who did not engage in regular exercise (Figure 15).
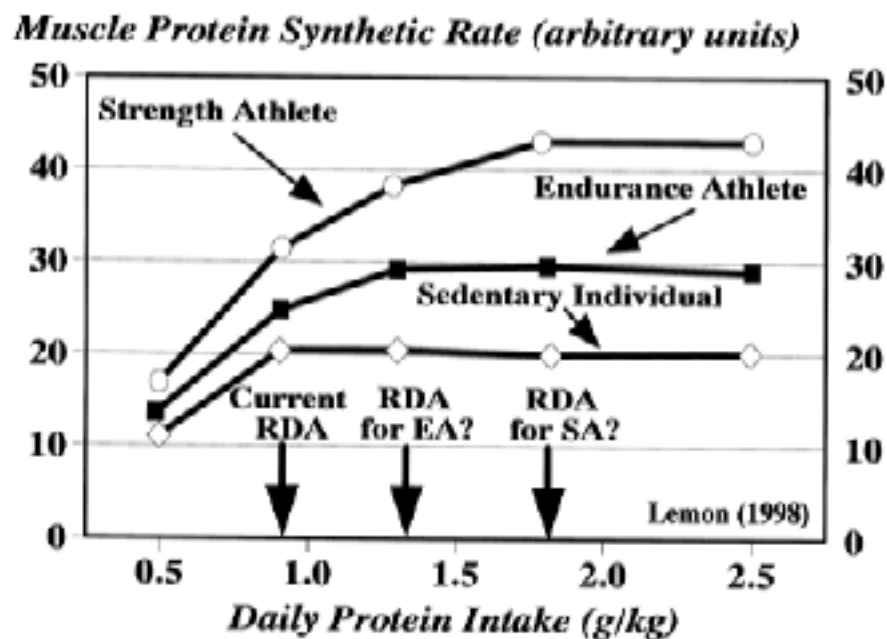
Figure 15. Schematic Representation of How Exercise May Alter Protein Requirements. *Data from* Lemon 1998.

Although not always appreciated, it is possible that, even if a measure like nitrogen balance does not indicate an increased protein requirement, exercise performance could still be enhanced by a greater protein intake, i.e., the additional protein might alter a metabolic process enhancing energy utilization for endurance exercise or could stimulate anabolism resulting in greater muscle mass and/or strength gains (Lemon 2000a).

In this thesis, careful attention must be paid to semantics. It is important to keep in mind that the words "need" or "requirement" as they frequently appear in this paper does not simply refer to the minimum amount of dietary protein necessary to sustain health, but to the amount of protein necessary to optimize exercise performance, while not compromising other dietary or health aspects.

3.2   Type of exercise

There are many different kinds of exercise ranging from high-intensity, short-duration activity (strength or speed exercise) to moderate-intensity, long duration activity (endurance exercise). Although the acute response to exercise

at either extreme of this exercise continuum follows a similar pattern (decreased protein synthesis and increased protein degradation during and immediately following exercise [Booth and Watson 1985]), the time course and absolute magnitude of these component responses clearly differ. As a result, the overall effect of chronic exercise at one end of this exercise continuum is vastly different from the other.

With endurance exercise, there are dramatic increases in mitochondrial (enzymatic) protein with minimal effects on muscle mass and strength (myofibrillar protein) while with strength or speed exercise, there is little change in mitochondrial protein but the gains in muscle mass and strength are truly phenomenal (**Table 2**).

Table 2. Adaptive Changes in Skeletal Muscle with Regular Bouts of Exercise

Type of Exercise	Muscle size (myofibrillar content)	Myofibrillar concentration	Cytochrome c content	Cytochrome c concentration
Running	→	→	↑	↑
Swimming	↑	→	↑	↑
Weight-lifting	↑	→	↑	→

Data from Booth and Watson 1985

For this reason, the underlying rationale for increased protein need with different types of exercise may be substantially different (Lemon 1992b). For example, in situations where energy demands are high and especially when prolonged, protein may provide a significant quantity of amino acids for use as an auxiliary exercise fuel. If so, inadequate dietary protein and/or too frequent training (overtraining) could lead to losses of endogenous protein (probably both liver and muscle) and eventually to impaired performance.

In contrast, when attempt is made to increase cell mass (hypertrophy), additional dietary protein may be necessary to provide sufficient amino acids to maximize protein synthesis. As with endurance training, strength or speed training can also be done too frequently. In this case, the exercise catabolic response exceeds the subsequent anabolic response and the overall result is

either reduced gains or even decreases in muscle mass and strength. Perhaps additional dietary protein can minimize this effect and, therefore, produce greater exercise-induced hypertrophy.

In addition, some degree of muscle soreness is frequently present following exercise, especially with eccentric exercise, and both structural changes in muscle (mitochondrial swelling, degenerating fibres, contractile protein disorientation, etc.) and efflux of muscle-specific enzymes into the vascular system have been reported (Berg and Haralambie 1978; Janssen et al. 1989; Kuipers et al. 1989). Additional dietary protein might help promote the recovery/repair process and as a result improve exercise performance.

3.3   Protein requirements of endurance athletes

3.3.1   Studies reporting increased protein needs with endurance exercise

Utilizing the balance technique, Gontzea et al. (1974, 1975) observed that, at least for the first 20 days of an endurance training programme, daily protein needs were elevated to approximately $1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. In agreement with these data are a number of Japanese studies which found that a protein intake of approximately $2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ could reduce the observed loss of blood proteins during the first week or two of daily exercise (Yoshimura et al. 1980).

Moreover, data from experiments with experienced athletes (2-40 years of training) from several different laboratories agree that the increased need continues long after the first weeks of training. In one study, Tarnopolsky et al. (1988) reported that a protein intake of $1.37 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ was necessary to maintain nitrogen balance with trained endurance men in their early twenties.

Based on data from similar study, Meredith et al. (1989) suggested a protein intake of $1.26 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for men (23-59 years) who participated in regular endurance exercise. In male runners (24-29 years) who trained regularly for years, Friedman and Lemon (1989) calculated protein requirements to be between 1.14 and $1.39 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$.

Finally, Brouns et al. (1989a, 1989b) observed that well-trained long distance cyclists (simulated Tour de France competition) needed a protein intake in the range of 1.5-1.8 g $\cdot$ kg^{-1} $\cdot$ day^{-1}.

3.3.2 Other lines of evidence that protein needs are increased with endurance exercise

In addition to these nitrogen balance studies several other lines of evidence indicate that dietary protein needs are increased with regular endurance exercise. For example, a number of studies have reported substantial increases in whole body oxidation of the branched chain amino acid leucine (for references see Lemon 1992b). Furthermore, numerous studies have measured an increased concentrations (body and/or tissues) or excretion (urine and/or sweat) of nitrogen (waste product of protein utilization) associated with prolonged exercise (for references see Lemon 1992b).

3.3.3 Studies reporting no increased protein needs with endurance exercise

Other researchers who have examined the issue of protein requirements in exercising humans have concluded, however, that endurance exercise does not elevate the requirement for dietary protein (Butterfield and Calloway 1984; Todd et al. 1984; Butterfield 1987). In the latter studies, it was shown that exercise actually improves the economy of nitrogen (protein) utilization; however, high quality egg protein was being ingested and all subjects were meticulously monitored to ensure they were in energy balance (Phillips 2002).

A diet based entirely on egg protein differs from that used in previous studies in which protein was from mixed sources, which would likely be more reflective of what an athlete habitually ingests. Further, in at least two of the previous studies, the athletes were checked to ensure that they were weight-stable, meaning that energy balance must have been sufficient (Phillips 2002).

3.4 Protein requirements of strength athletes

3.4.1 Theoretical calculations of the protein required for muscle tissue synthesis

If we take the theoretical example of a person who initially weights 100 kg and in a given year gains 10 kg of muscle (it needs to be strongly emphasized that this gain is purely muscle and just not body mass), this represents a highly impressive gain of lean muscle mass and likely at the "outer limit" of possible gains in lean body mass, without anabolic steroids. The question is, how much extra protein would this individual have to consume? According to Phillips (2002),

1. 10 kg muscle = 2.5 kg protein (assuming 75% of muscle is water.)

2. Then 2.5 kg protein = 2 500 g in one year or 2 500 g/365 d/100 kg =0.0685 g protein kg day that is gained.

3. Assuming that, based on some values calculated from growing steers, *eight* times as much protein needs to be consumed to lay down the same amount of mass (note, this mass gain is not all muscle in steers and so application of this value to humans represent an overestimate): $0.0685 \cdot 8 = 0.55$ g protein $\cdot$ kg$^{-1} \cdot$ day^{-1}.

4. Assuming that the RNI/RDA/DRI is sufficient to cover all other protein needs, 0.86 g protein $\cdot$ kg$^{-1} \cdot$ day^{-1} + 0.55 g protein $\cdot$ kg$^{-1} \cdot$ day^{-1} = 1.41 g protein $\cdot$ kg$^{-1} \cdot$ day^{-1}.

What this calculation does not take into account is that resistance exercise actually increases the efficiency of protein and amino acid utilization (i.e., net muscle protein balance is less negative), which would actually reduce the amount of protein required to gain the 10 kg of muscle (Phillips 2002).

Campbell et al. (1995) reported that 11 weeks of resistance training improved nitrogen balance by approximately 13 mg N $\cdot$ kg^{-1} $\cdot$ day^{-1} or 82 mg protein $\cdot$ kg^{-1} $\cdot$ day^{-1}, which would reduce the estimated dietary protein requirement of 1.41 g protein $\cdot$ kg^{-1} $\cdot$ day^{-1} to 1.33 g protein $\cdot$ kg^{-1} $\cdot$ day^{-1}. However, it is possible that additional protein could stimulate anabolism resulting in greater muscle mass and/or strength gains.

3.4.2   Studies reporting increased protein needs with strength exercise

Celejowa and Homa (1970) reported a negative nitrogen balance in at least 40% of male weight lifters consuming protein equal to approximately 2 g $\cdot$ kg^{-1} $\cdot$ day^{-1}. Larictheva et al. (1978) found that protein intakes of 1.3.-1.6 g $\cdot$ kg^{-1} $\cdot$ day^{-1} were necessary to avoid negative nitrogen balance.

Torun et al. (1977) observed a decreased cell mass (based on ^{40}K measures) over 6 weeks of strength training when protein intake was approximately 70 to 100% of current recommendations.

More recent data indicate that protein needs of male bodybuilders are approximately 1.2 to 1.7 g $\cdot$ kg^{-1} $\cdot$day^{-1} (Tarnopolsky et al. 1988, 1990; Lemon et al. 1990, 1992a) (**Figure 15**).
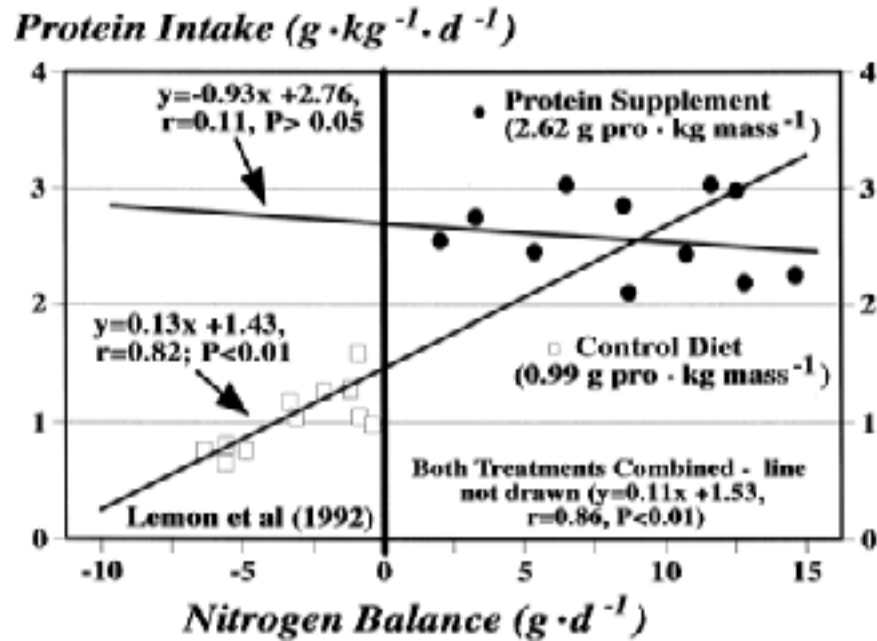
Figure 15. **Effect of Quantity of Dietary Protein on Nitrogen Balance in Novice Bodybuilders.** Note that for protein intakes around 1.0 g · kg^{-1} · day^{-1} (solid squares) the Y intercept (dietary protein requirement) is 1.43 g · kg^{-1} · day^{-1} (more than double the sedentary requirement). Note also that with high protein intakes (~2.6 g · kg^{-1} · day^{-1}, filled circles) the linear relationship between nitrogen retention and protein intake does not hold up. *Data from* Lemon et al. 1992a.

Although these studies clearly suggest that protein needs of strength or speed athletes exceed current recommendations the absolute values (1.2-2.0 g · kg^{-1} · day^{-1}) fall considerably below the intakes reported by many strength athletes, especially bodybuilders. This discrepancy could be explained if nitrogen balance measures are insufficient to completely assess the value of high-protein diets (Lemon 1992b). *These athletes are not concerned with nitrogen balance but rather on their absolute gains in muscle mass and strength.*

Oddoye and Margen (1979) have demonstrated that it is possible to maintain highly positive nitrogen balance for long periods of time (at least 50 days) when protein intake is very high (approximately 300% of current recommendations). Perhaps a highly positive nitrogen balance when combined with powerful anabolic stimulus of heavy resistance exercise can enhance protein synthesis and/or reduce protein degradation resulting in greater gains in mass and strength (Lemon 1992b).

Although far from conclusive some experimental data are consistent with this hypothesis. For example, Consolazio et al. (1975) observed greater nitrogen retention (32.4 vs. 7.1 g) and greater gains in lean body mass (3.28 vs. 1.21 kg) over 40 days of training when protein intake was 2.8 vs. 1.4 g $\cdot$ kg^{-1} $\cdot$ day^{-1}.

Marable et al. (1979) reported greater nitrogen retention in men during a strength programme while consuming protein equal to approximately 200-300 vs. 67-100% of current recommendations. In Romanian weight-lifters, Dragan et al. (1985) found gains in strength (5%) and lean body mass (6%) with several months of strength training when protein intake was increased from 2.2 to 3.5 g $\cdot$ kg^{-1} $\cdot$ day^{-1}.

In addition, Lemon et al. (1990) and Tarnopolsky et al. (1990) also observed a greater nitrogen retention (**Table 3**) on a protein intake of 2.67 vs. 0.99 g $\cdot$ kg^{-1} $\cdot$ day^{-1} in novice bodybuilders during intensive training.

Table 3. Nitrogen Balance During One Month of Strength Training with Variable Protein Intake

Nitrogen balance[a]	Group 1	Group 2
Intake (g $\cdot$ day^{-1})	12.8 ± 0.9	34.8 ± 1.3[b]
Excretion (g $\cdot$ day^{-1})		
Urine	12.5 ± 0.3	21.1 ± 1.6[b]
Feces	2.0 ± 0.3	2.2 ± 0.3
Sweat	1.6 ± 0.1	2.5 ± 0.1[b]
Balance (g $\cdot$ day^{-1})	-3.4 ± 0.5	8.9 ± 1.2[b]

Data from Lemon et al. 1990; Tarnopolsky et al. 1990.
[a]Values are means ± S.E. for 12 subjects/group.
[b]$P < 0.01$ between groups.

Finally, Tarnopolsky et al. (1992) addressed this issue by comparing measured rates of whole-body protein synthesis for strength athletes in regular training with sedentary controls while both groups consumed protein at each of three different protein intake (0.9, 1.4, and 2.4 g $\cdot$ kg^{-1} $\cdot$day^{-1}).

Although increasing protein intake did not affect protein synthetic rates in the control, increasing from 0.9 to 1.4 g · kg^{-1} · day^{-1} produced a significant increase in protein synthesis in the strength athletes (**Figure 16).** This is a significant observation because over time this acute increase in protein synthesis would be expected to result in increased muscle size and strength. Consequently, this is objective evidence supporting the athletes` opinions (i.e., dietary protein in excess of the RDA in combination of with strength training can enhance muscle growth).

However, of equal importance was the second finding of this study. The consumption of even greater amounts of protein (2.4 g · kg^{-1} · day^{-1}) did not further increase the protein synthetic rate in strength athletes.
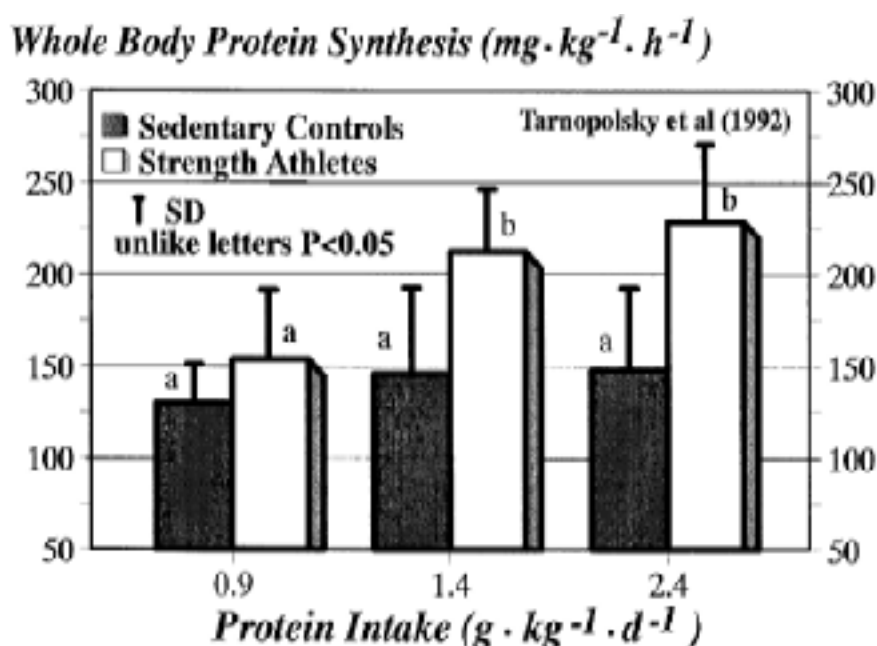
Figure 16. Effect of Quantity of Dietary Protein on Whole Body Protein Synthetic Rate. Note that increasing dietary protein above the requirement for sedentary people (0.8 g ·kg^{-1} · day^{-1}) had no effect on protein synthesis; however, increasing from 0.9 to 1.4 g · kg^{-1} ·day^{-1} in the strength athletes led to an increase in protein synthesis. Over time this should increase both muscle mass and size. Note also that a further increase in protein intake (to 2.4 g · kg^{-1} · day^{-1}) did not increase protein synthesis any further indicating that this was more dietary protein than needed. *Data from* Tarnopolsky et al. 1992.

However, as Rennie et al. (1994) pointed out, there were no detectable effects of varying protein intake on 24-hr creatinine excretion (i.e., an index of muscle mass) or of lean body mass. These results occurred despite differences

in nitrogen balance during 13-day periods of low- and high-protein intake of approximately 6 g nitrogen $\cdot$ day^{-1} equivalent to nearly 2 kg of lean tissue over 13 days.

Authors suggest that their measures may simply not have been sufficiently sensitive to detect any such changes, According to Rennie et al. (1994), this is difficult to accept because the limits of detection was approximately 1.3 kg lean body mass in the strength-trained subjects.

Fern et al. (1991) compared protein intakes in bodybuilders assigned to either 3.3 vs. 1.3 $\cdot$ kg^{-1} $\cdot$ day^{-1}. Results showed significantly greater gains in body mass over 4 weeks of training at the higher protein intake. Metabolic tracer data indicated that protein synthesis increased with training regardless of diet; however, with the higher protein intake the increase in synthesis was fivefold greater.

This observation is of considerable important because it appears to be first documentation that a protein intake of approximately four times the RDA, in combination with strength training, can promote greater muscle size gains than the same training with a diet containing adequate protein.

Unfortunately, amino acid oxidation also increased 150% on the higher intake, which suggested that the optimum protein intake was also exceeded (Lemon 2000b).

3.4.3   Studies reporting no increased protein needs with strength exercise

Nitrogen balance studies conducted in the elderly have shown that initiating a moderate program of strength training resulted in reduced protein requirements due to the anabolic stimulus of the resistance exercise (Campbell et al. 1995). However, even following 10 weeks of comparatively mild resistance exercise training, there was no evidence of muscle hypertrophy in people consuming either 0.8 or 1.6 g protein $\cdot$ kg^{-1} $\cdot$ day^{-1}.

The results of Campbell et al. (1995) are remarkably similar to those reported by Torun et al. (1977) showing that isometric exercise improved protein utilization. Hence, while resistance exercise did improve nitrogen balace, the results of Campbell et al. (1995) and Torun et al. (1977) may not be directly applicable to younger resistance training athletes who are trying to gain lean mass and stimulate hypertrophy (Phillips 2002).

That the athletes studied by Tarnopolsky et al. (1988, 1992) were all highly trained at the time of study and were performing exercise that was more intense than that described in the studies of Campbell and Torun that showed a reduction in protein requirements, may be a reason for the discrepancy (Phillips 2002).

3.4.4   Importance of drug-free subjects

The importance of drug-free subject for these studies cannot be overemphasized. It is widely recognized by those familiar with the sports of bodybuilding and weightlifting that a surprising number of bodybuilders, especially those who compete, self-administer a number of prescription drugs including anabolic-androgenic steroids.

It has been estimated that 90% of male professional bodybuilders use anabolic-androgenic steroids at any given time (Catlin et al.1993). Anabolic androgenic steroids with their profound affect on protein synthesis and degradation could affect nitrogen balance studies (**Table 4**).

Table 4. Comparison of Steroid Protein Anabolic Index (SPAI)[a] of Some Newer Oral Steroids in Convalescent Adults

Steroid	Number of assays	Dosage mg · day^{-1}	Average SPAI
Testosterone propionate	12	10-25	+6
19-nortestosterone	12	25-75	+9
Oxandrolone	27	10-20	+20
Methandrostenolone	16	5-30	+16
Stanozolol	10	6-12	+29
Norbolethone	6	7.5-10	+34

Modified from Kochakian and Yesalis 2000.
[a]Steroid anabolic index = NBSP / NISP – NBCP / NICP x 100
NBSP = nitrogen balance in steroid period; NISP =nitrogen intake in steroid period; NBCP = nitrogen balance in control period; NICP = nitrogen intake in control period.

As pointed out by Bradley-Popovich (1999), it is very probable that steroids were used among the competitive subjects in the studies of Celejowa and Homa (1970) and Laritcheva et al. (1978). On the contrary, it is extremely unlikely that the older neophytes in Campbell's (1995) study would have used physique-enhancing drugs.

4   Factors which appear to affect dietary protein need

4.1   Dietary energy

Is has been know for about a half century that inadequate energy intake leads to increased protein needs, presumably because some of the protein normally used to synthesize both functional (enzymatic) and structural (tissue) protein is utilized for energy under these conditions.

Apparently, this effect on protein need is similar when the energy deficit is caused by increased energy expenditure (exercise). In fact, this effect could be even more dramatic in those who are physically active, as protein needs are likely already increased in order to maintain a greater protein synthetic rate due to the presence of greater absolute tissue (strength athletes) or enzyme (endurance athletes) levels.

In addition, there appears to be a gender difference in one´s ability to increase food intake adequately with chronic high-intensity exercise. Perhaps for reasons related to maintenance of reproductive functions in times of energy deficit, females are better able to reserve functional tissue than males whenever energy intake is low (Lemon 2000a).

Although this is of obvious benefit in a starvation situation, for physically active females it often results in under eating relative to energy expenditure (**Figure 17**).
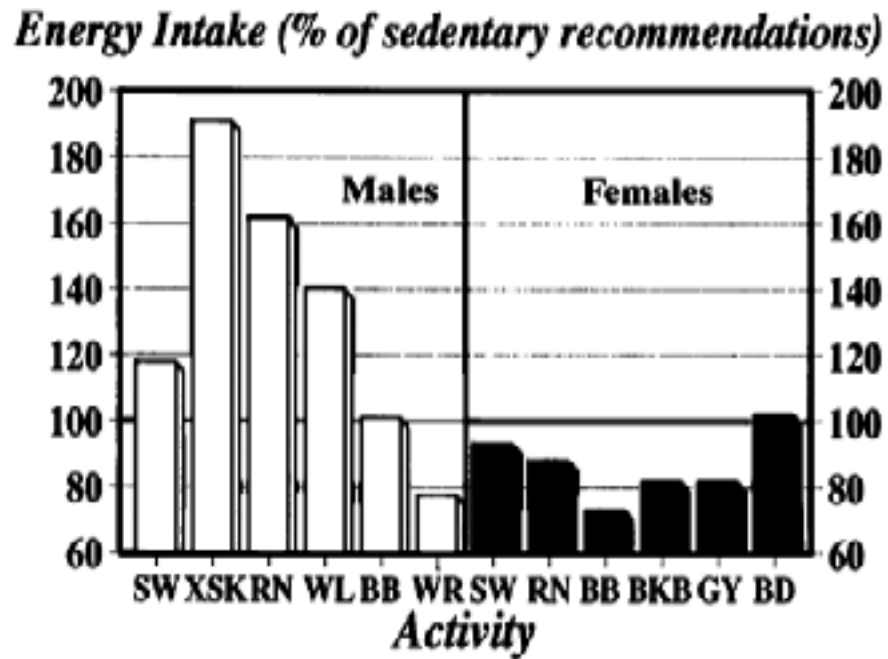
Figure 17. **Gender Effects on Energy (Food) Intake in Physically Active Individuals.**
SW = swimmers; XSK = cross-country skiers; RN = distance runners; WL = weight lifters; BB = bodybuilders; WR = wrestlers; BKB = basketball players, GY = gymnasts; BD = ballet dancers. While men typically increase their energy intake appropriately for the increased expenditure of their activity (with the exception of bodybuilders and wrestlers), women routinely fail do to so. *Data from* Lemon 2000a.

Butterfield (1987) has shown that ***feeding as much as 2 g protein $\cdot$ kg^{-1} $\cdot$ day^{-1} to men running 5 or 10 miles per day at 65% to 75% of their VO$_{2max}$ is insufficient to maintain nitrogen balance when energy intake is inadequate by as little as 100 kcal $\cdot$ day^{-1}***.

4.2 Carbohydrate Content

Carbohydrate´s primary function is supplying energy for cellular work. Aerobic hydrolysis of carbohydrate for energy occurs more rapidly than energy generation from fatty acids. Thus, depleting glycogen reserves significantly reduces exercise power output. In prolonged aerobic exercises such as marathon running, athletes often experience nutrient-related fatigues – a state associated with muscle and liver glycogen depletion.

Inadequate carbohydrate for muscle contraction is also critical because its availability is inversely related to the rate of exercise protein catabolism (**Figure 12**). Therefore, daily carbohydrate intake is of great significance for physically active individuals.

Moreover, physically active individuals need to be much less concerned about excess dietary carbohydrate intake resulting in surplus body fat storage compared to their sedentary counterparts because this substrate is used to replenish carbohydrate stores depleted by exercise training/competition sessions.

According to Jonnalagadda (2002), athletes undergoing prolonged (> 60 min) intense training (65-70% VO_{2max}) require 8-10 g carbohydrate $\cdot$ kg^{-1} $\cdot$ day^{-1}, while those training less than 60 min day^{-1} require 5 g carbohydrate $\cdot$ kg^{-1} $\cdot$ day^{-1}.

4.3   Protein quality

4.3.1   Basic facts about protein quality

Not all proteins are of equal nutritional value; this reflects their differing amino acid content. Although most proteins contain most of the 20 or so amino acids, these are present in widely differing proportions. Complete proteins contain all of the essential amino acids in sufficient amounts to maintain life and support growth in that animal. Partially incomplete proteins can maintain life, but cannot support growth.

Since plant proteins may be deficient in one or more essential amino acids and meat contains all of the essential amino acids, the chances of developing a deficiency is greater for vegetarians than for meat eaters. A lack of essential amino acids in the diet results in a variety of adverse effects that depend on the degree and length of deficiency. Dairy products, meat, fish, eggs, and poultry are examples of foods that contain complete proteins.

Essential amino acids are those which cannot be synthesized by the body or that cannot be synthesized at a sufficient rate to supply the normal requirements for protein biosynthesis. The nonessential amino acids can be synthesized at a sufficient rate (provided, or course, that the supplies of amino nitrogen and carbon precursors are adequate). Thus, an amino acid is nonessential if its carbon skeleton can be formed in the body, and if an amino group can be transferred to it from some available donor compound.

The use of word essential, however, does not mean that the other nonessential amino acids are not equally as essential for formation of the proteins, but only that the others are not essential in diet because they can be synthesized in the body. For protein synthesis to take place, all the amino acids required must be available. If the diet lacks one or more of these essential amino acids the body´s ability to synthesize new protein is adversely affected (Di Pasquale 1997).

4.3.2   Measuring protein quality

There are several ways to measure the protein quality of food. The three most quoted are the *1)* Protein Efficiency Ratio (PER), *2)* Biological Value (BV), and *3)* Protein Digestibility-Corrected Amino Acid Score (PDCAAS).

4.3.2.1   Protein Efficiency Ratio (PER)

In PER procedure, immature rats are fed a measured amount of protein and weighed periodically as they grow. The PER is then calculated by dividing the weight gain (in grams) by the protein intake (also in grams).

However, evidence from studies with rats indicate that the pattern of amino acids required for maintenance and tissue protein accretion is quite different (Di Pasquale 1997). Thus, the amino acid requirements of growing rats are not the same as for those of mature rats, much less mature human beings.

Indeed, the intracellular muscle free amino acid pool of rats is probably less suitable for the investigation of amino acid metabolism, due to the great differences in their distribution in human and rat muscle (Furst 1985). Nevertheless, the PER is used widely today.

Hernandez et al. (1996) compared the protein quality of different animal foods and of their mixture with vegetable foods, mainly cereals, at the 30:70 animal:vegetable protein proportions with experiments performed under the same conditions. The animal foods were eggs, beef, pork. barbecued lamb, chicken, ham, sausage, and milk powder. The vegetable foods used in the mixtures were rice, lime-treated corn flour, wheat flour, and cooked black beans. The protein concentrations in the raw and cooked materials were analyzed. The PER and digestibilities were determined in Fisher 344 weanling rats.

Based on the corrected PER, the foods with the best protein quality were egg (3.24), sirloin beef (3.16), lamb (3.11) and chicken breast (3.07), which were significantly different from milk powder (2.88) and beef liver and beef round (2.81 and 2.70, respectively). Ham (2.63) and pork loin (2.57) had a similar quality to that of casein (2.50). The lowest protein quality was found in sausages (2.14). In most of the mixtures of animal and vegetable protein (30:70), the PER was similar to or higher than of the animal food alone. Beans were the vegetable food that showed the lowest response to the addition of animal protein.

We have recently compared PER values of commercial protein powders: *1)* bovine colostrum + standard rat diet 50:50, *2)* calcium caseinate + standard rat diet 50:50, and *3)* standard rat diet (Manninen AH and Leppäluoto J, unpublished data). The results indicate that bovine colostrum supports growth more effectively than either calcium caseinate or standard diet (**Table 5**).

Table 5. Protein Efficiency Rations (PER) of Bovine Colostrum (BC)[a], Calcium Caseinate (CAS) and Standard Diet (STA)

Group	Number	Mean PER	Std Dev	SEM
BC + STA 50:50	7	1.546	0.140	0.0530
CAS + STA 50:50	7	0.624	0.0336	0.0127
STA	6	1.192	0.0523	0.0214
Comparison	Diff of Means	p	q	P
BC vs. CAS	0.921	3	26.997	< 0.001
BC vs. STA	0.354	2	9.966	< 0.001
STA vs. CAS	0.567	2	15.971	< 0.001

[a]Bovine colostrum is the first milk secreted by cows after parturation and contains a number of bioactive proteins, including growth factors, of which insulin-like growth factor is one of the most abundant and well-characterized.
Unpublished data from Manninen AH and Leppäluoto J, Department of Physiology, University of Oulu, Finland, 2002.

However, it should be noted that PER values in our study were significantly lower than reported by Hernandez et al. (1996). Further, it is not known what underlying mechanism(s) is responsible for significantly higher PER results in bovine colostrum group.

4.3.2.2   Biological Value (BV)

BV is calculated as retained nitrogen/absorbed nitrogen x 100 (for detailed review see Di Pasquale 1997). The BV method has advantages of being based on experiments with human beings and of measuring actual nitrogen retention. Its disadvantages are that it is cumbersome, expensive, sometimes impractical, and is based on several assumptions that may not be valid.

For example, the subjects used for testing may not be similar physiologically or in terms of their normal enviroment or typical food intake (e.g., dietary protein intake) to those for whom the test protein may ultimately be used. Also, protein retained in the body does not necessarily mean that it is being well utilized (Di Pasquale 1997).

4.3.2.3   Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

The PDCAAS method is a simple and scientifically sound approach for routine evaluation of protein quality of foods. It is directly applicable to humans, and incorporates factors and more real-life variables than either PER or BV.

The amino acid pattern for humans aged 2 to 5 years is used as the basis for the determination of PDCAAS, since this age group matches or exceeds amino acids requirement pattern of older children and adults (Di Pasquale 1997) Corrections for digestibility of protein are also taken from human data. PDCAAS scores range from 1.0 to 0.0, with 1.0 being the upper limit of protein quality (**Figure 18**).
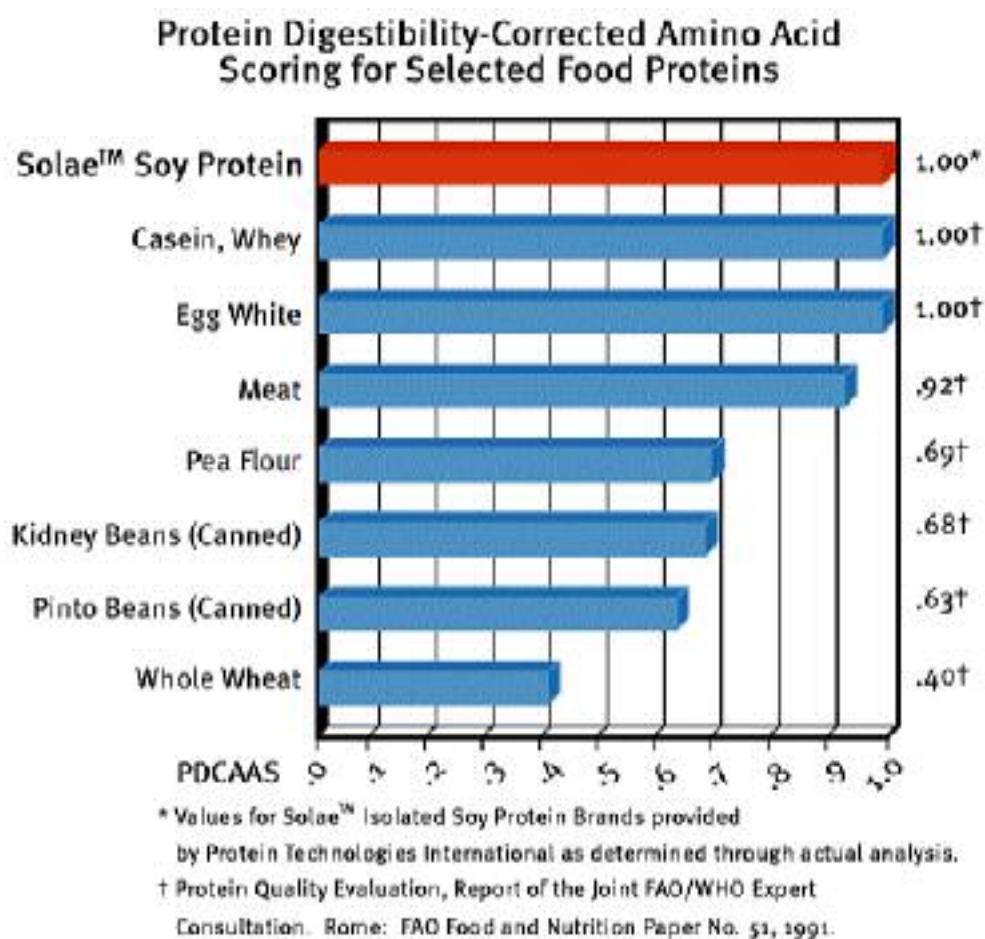
Figure 18. Comparison of Protein Quality (PDCAAS). Solae™ refers to isolated soy protein. *Data from* Protein Technologies International.

4.4   Gender

4.4.1   Gender differences in endocrine function

Males have negligible concentrations of circulating estrogen and progesterone. Conversely males have higher concentration of testosterone. These comparisons highlight the large endocrinological differences in between males and females, and also indicate the dramatic variation in endocrine function during the menstrual cycle of the female.

There are three phases within the menstrual cycle: *1)* the follicular phase, which begins with the onset of menstruation and has a variable length of between 9 to 23 days; *2)* the ovulatory phase, which results in the release of the ovum and may last 3 days; and *3)* the luteal phase, which extends from ovulation to the onset of menstrual bleeding. The luteal phase is generally more consistent in duration, lasting approximately 13 days.

The distinct phases of the menstrual cycle are important because there are differences in hormone concentrations during these phases that are influential in regulating fuel mobilization during exercise.

The naturally occurring estrogens are 17β-estradiol, estrone, and estriol. They are secreted primarily by the granulose cells of the ovarian follicles, the corpus luteum, and the placenta. 17β-estradiol is the most potent of estrogen of the three, and estriol the least. The estradiol secretion rate is 36 $\mu g \cdot day^{-1}$ in the early follicular phase, 380 $\mu g \cdot day^{-1}$ just before ovulation, and 250 $\mu g \cdot day^{-1}$ during the midluteal phase (Ganong 2001d).

Progesterone is secreted by the corpus luteum, the placenta, and the follicle. The plasma progesterone is approximately 0.9 $ng \cdot mL^{-1}$ during the follicular phase of the menstrual cycle (Ganong 2001d). Late in the follicular phase, progesterone secretion begins to increase. During the luteal phase, the corpus luteum produces large quantities of progesterone and there is marked increase

in plasma progesterone to a peak value of approximately 18 ng · mL^{-1} (Ganong 2001d).

4.4.2   Gender differences in protein metabolism

4.4.2.1   Whole body protein metabolism

During endurance exercise leucine oxidation is increased in both males and females (Tipton 2001). Most authors report that leucine oxidation is greater in males than in females during exercise (Phillips et al. 1993; McKenzie et al. 2000; Lamont et al. 2001).

Only one study has reported no differences in leucine oxidation between males and females during exercise (Bowtell et al. 1999), but only two females were studied, leaving the results somewhat questionable due to possibility of a type II error.

Lariviere et al. (1994) demonstrated that leucine oxidation was 18% greater during the luteal phase than the follicular phase in female study participants. Thus, females studied in the luteal phase may have leucine oxidation levels that are closer to those of the males.

Consistent with this notion, Phillips et al (1993) reported a 77% difference and McKenzie et al. (2000) a 115% difference between males and females studied only in the mid-follicular phase. In contrast, Lamont et al. (2001) found no difference in females, six out of ten of whom were studied in the follicular phase, and males.

4.4.2.2   Muscle protein metabolism

It is clear that there are differences in musculature between adult male and female humans. Muscle mass, especially upper body mass is greater in males

than females (Tipton 2001). Muscle fiber distribution is similar, but the cross-sectional area of muscle fibers is greater in males than females (Tipton 2001).

The metabolic basis for larger muscle is positive net muscle protein balance, that is, muscle protein synthesis must exceed muscle protein breakdown. Thus, males must exhibit positive muscle protein balance to a greater degree than females, at least at some point in development.

4.4.2.3 Testosterone and gender differences in protein metabolism

The testosterone secretion rate is 4-9 mg $\cdot$ day^{-1} in normal adult males (Ganong 2001d). Small amount of testosterone are also secreted in females, probably from the ovaries but possibly from the adrenals as well. The plasma testosterone level (free and bound) is 300-1000 $\cdot$ ng dL^{-1} in adult men and 30-70 ng $\cdot$ dL^{-1} in adult women (Ganong 2001d).

Until puberty, boys and girls are similar in size and muscle mass (Tipton 2001). During puberty, boys increase muscle mass to greater extent than girls. At the same time, testosterone levels increase in the boys, but not in the girls (Tipton 2001).

These facts strongly suggest that testosterone plays a role in the obvious differences in muscle between males and females, *but little direct evidence is available*. Data on testosterone administration in females are necessarily limited, due to practical and ethical considerations.

4.4.2.4 Ovarian hormones and gender differences in protein metabolism

There is much less information on the role that ovarian hormones play in muscle protein metabolism. No study has been performed on humans *in vivo*, but there is information from rats and in-vitro studies.

In a recent study, accumulation of fat-free mass was greater in ovariectomized rats than in ovariectomized rats given estrogen and progesterone replacement (Toth et al. 2001). Furthermore, muscle protein synthesis in the ovariectomized rats was greater than sham-operated rats, as well as the ovariectomized rats in which the ovarian hormones had been replaced. These results suggest that ovarian hormones inhibit muscle protein synthesis. In-vitro studies support the notion that estrogen inhibits muscle protein synthesis (Roeder et al. 1986; Desler et al. 1996).

Thus, *whereas it seems that increasing levels of testosterone seem to contribute to increased muscle mass in males by increasing muscle protein synthesis and decreasing muscle protein breakdown, ovarian hormones may attenuate muscle growth in females by inhibiting muscle protein synthesis.* Clearly, this is an interesting concept that should be carefully examined *in vivo* in human subjects.

4.5 Age

Sarcopenia is a term used to describe the loss of skeletal muscle mass with advancing age. Although part of this muscle loss is likely the result of reduced activity, physiological/biochemical processes are also involved, as indicated by the 30% reduction in myofibrillar protein synthesis in individuals over 60 years of age (**Figure 19**).
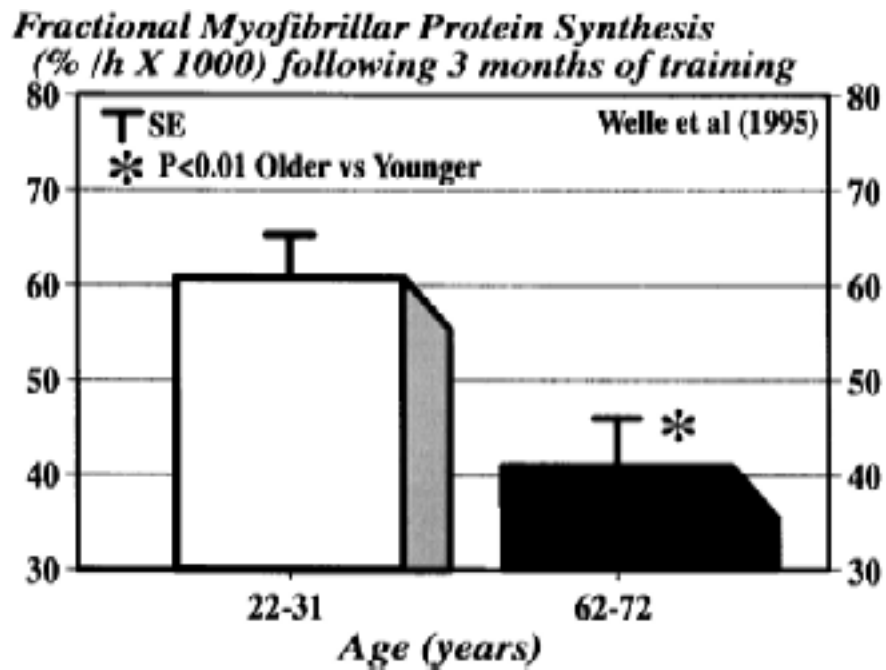
Figure 20. **Effect of Age on Muscle Protein Synthesis**. Myofibrils are numerous threadlike structures that contain the contractile proteins. In general, myofibrils are composed of two major types of protein filaments*: 1)* thick filaments composed of protein myosin and *2)* thin filaments composed primarily of the protein actin. These proteins make up only a small portion of the muscle, but they play an important role in the regulation of the contractile process. *Data from* Welle et al. 1995.

Further, muscle performance/function improves with strength training even into the tenth decade of life, and this not due to improved neurological function alone, as three months of regular strength exercise can increase mixed muscle protein synthesis even in the frail elderly (76 to 92 years-old men and women) (Lemon 2000a).

Typically, nutrient intake is less than ideal in the elderly, and although short term (10 day) energy and protein supplementation can enhance protein synthesis and fat-free mass in 60 to 90 year-old men and women, whether nutritional supplementation might enhance further muscle growth with strength exercise in the elderly is an interesting possibility.

One study observed that a 360 kcal (60% carbohydrate, 23% fat, 17% protein) supplement in combination with a 10-week strength program increased both muscle strength and size more than the same training without supplementation in 72 to 98 year-old men and women (Fiatarone Singh et al. 1999).

In contrast, acute (one-day) feeding of protein at 0.6, 1.2, or 2.4 g · kg^{-1} did not affect myofibrillar protein synthesis following very brief (three-session) knee extension program (Welle and Thornton 1998).

The other end of the age continuum is also of interest because dietary protein needs are know to be greater due to growth (US Food and Nutrition Board 1989). Although not systemically investigated, it is possible that regular physical activity could further increase protein requirements for this population (Lemon 2000a).

5 Recommended protein intakes for physically active individuals

Taken together the weight of the existing evidence strongly indicates that daily protein needs are increased as a result of either regular strength-speed or endurance training. **Table 6** presents a summary of the recommended daily protein intakes for physically active individuals.

Table 6. Recommended Protein Intakes (g · kg body mass^{-1} · day^{-1}) for Physically Active Individuals

Strength-trained, maintenance	1.2-1.4
Strength-trained, gain muscle mass	1.6-1.8
Endurance-trained	1.2.-1.4
Weight-restricted	1.4-1.8

Data from Williams 1999. The values presented represent a synthesis of those recommended by leading researchers involved in protein metabolism and exercise. Teenagers should add 10% to the calculated values.

However, the testimonials of athletes who believe that their success depends on consumption of large amounts of protein and energy, and the examples of Japanese sumo wrestlers and Olympic weight lifters, suggest that further laboratory investigations are necessary before the question of protein need in those attempting to increase lean mass is settled.

Several possibilities might explain this apparent contradiction. Obviously, the athletes could be incorrect, i.e., they may have been influenced by a powerful placebo effect. Alternatively, some other constituent(s) in high-protein foods might, in combination with surplus supply of amino acids, be responsible for a muscle-building effect. Several candidates are possible including creatine, a nitrogen compound found in meat and fish that has been studied recently (see *11.3. Effects of nutritional supplements designed to promote lean tissue accretion*).

Finally, although associated with a variety of adverse health effects, some compounds (i.e. anabolic steroids) are know to be anabolic and it is possible

that the high-protein intakes consumed by some strength athletes are only advantageous when combined with these agents (Lemon 2001).

5.1 Habitual protein intakes

Tables 7 and 8 show, respectively, the reported habitual dietary protein intakes of persons engaged in regular endurance activities (running, cycling and triathlon training) and resistive training.

Table 7. Reported Habitual Protein Intakes for Endurance Athletes

Reference	N	Intake $(g \cdot kg^{-1} \cdot d^{-1})$
Am J Clin Nutr 44, 954, 1986	51 F[a]	1.6 ± 0.6
New Engl J Med 311, 277, 1984	13 F (EM)	1.1 ± 0.5
	14 F (AM)	1.2 ± 0.5
Ann Intern Med 02, 158, 1985	6 F (EM)	1.3 ± 0.4
	11 F (AM)	1.0 ± 0.5
Am J Physiol 265, E380, 1993	17 F (EM)	1.0 ± 0.4
	11 F (AM)	0.7 ± 0.4
J Appl Physiol 75, 2134, 1994	6 M	1.9 ± 0.2
	6 F (EM)	1.0 ± 0.1
Int J Sports Med 10, S26, 1989	5 M	2.2 ± 0.5
J Appl Physiol 78, 1360, 1995	7 M	1.8 ± 0.4
	8 F (EM)	1.0 ± 0.2
J Appl Physiol 64, 187, 1988	6 M	1.5 ± 0.3
J Appl Physiol 83, 1877, 1997	8 M	1.9 ± 1.0
	8 F (EM)	1.2 ± 0.3
Means	32 M	1.8 ± 0.4[b]
	109 F (EM)	1.2 ± 0.3[c]
	36 F (AM)	1.0 ± 0.4[d]

Values are means ± SD. EM = eumenorrheic, AM = amenorrheic.
[a]Menstrual status not stated (assumed to be EM, unless otherwise indicated)
[b]14 ± 2% of total reported energy intake
[c]13 ± 3% of total reported energy intake
[d]15 ± 1% of total reported energy intake

Table 8. Reported Habitual Protein Intakes for Resistance-Trained Athletes and Bodybuilders

Reference	N	Intake $(g \cdot kg^{-1} \cdot d^{-1})$
J Appl Physiol 73, 1383, 1992	12 BB	1.6 ± 0.5
S Afr Med J 72, 831, 1987	76 BB	2.4 ± 0.6
Proc Nutr Soc 53, 223, 1994	26 BB[a]	1.9 ± 0.5
J Appl Physiol 73, 767, 1992	12 BB	1.4 ± 0.2
J Appl Physiol 82, 1882, 1997	10 RA	1.6 ± 0.2
J Am Diet Assoc 82, 632, 1983	30 RA	2.5 ± 0.8
	6 BB	2.3 ± 0.7
J Appl Physiol 64, 187, 1988	6 BB	2.7 ± 0.5
J Appl Physiol 73, 1986, 1992	7 RA	1.8 ± 0.4
Means	138 BB	$2.1. \pm 0.5$[b]
	47 RA	2.0 ± 0.4[c]

Values are means ± SD.
RA = resistance-trained athletes (includes football and rugby players).
BB = bodybuilders
[a]includes both males and females
[b]19 ± 3% of total reported energy intake
[c]18 ± 2% of total reported energy intake

As is obvious from both tables, all athletes are reportedly consuming in excess of all current recommended levels of protein intake including the Recommended Nutrient Intake (RNI; Canada), Recommended Dietary Allowance (RDA; USA), the World Health Organization and even the most recent Dietary Reference Intakes (DRI; Canada and USA).

The only group of athletes that may be at risk for suboptimal protein intakes, if one accepts that athletes´ reported dietary intakes of protein are accurate, might be female endurance athletes (see **Table 7**). Such a supposition should be cautionary, however, because almost all diet records or recalls appear to be subject to some degree of underreporting.

Estimates of underreporting range from 15%-30% of daily energy intake (Ballew and Killingsworth 2002). Women tend to underreport more than men, overweight individuals tend to underreport more than normal weight individuals and less educated individuals tend to underreport more than more educated individuals (Ballew and Killingsworth 2002).

5.2 Future directions for protein requirement studies

Some definitive studies, if properly conducted and controlled, might serve to clear up much of the controversy surrounding protein requirements in habitually active persons. The first study would be to have a group of highly trained endurance athletes having relatively high daily training volumes randomly consume protein at three different levels for prolonged periods of time (at least 3-4 weeks).

As in a traditional nitrogen balance experiment, protein would be consumed at a sub-adequate, adequate and more than adequate level. Each protein intake would have a period at the end of which measurements of protein kinetics, using appropriate stable isotope tracers, for muscle protein, plasma proteins (albumin, fibrinogen, fibronectin), as well as whole-body protein turnover and nitrogen balance. In addition, and most importantly from an athlete´s perspective, performance should be measured. Such studies have the following advantages (Phillips 2002):

- Studying previously habitually trained athletes so the transient negative nitrogen balance seen at training onset would not be a complicating factor.

- A prolonged period of adaptation to each diet to account for adaptations in enzymes involved in intramuscular protein metabolism and liver urea cycle enzymes.

- Measurement of protein kinetics for muscle, some blood proteins and at the whole body level, at each level of protein intake to account for adaptations in synthesis and turnover.

- Measurement of performance, which for athletes is the most important outcome.

A second study should include resistance-trained athletes. This study would have same advantages as outlined above. A third study should be planned to allow three comparatively many ($n = 20$ per group) of athletes who were inexperienced weightlifters to initiate a weightlifting program. Each group should have their diet manipulated so that they were consuming either 0.86 g protein $\cdot$ kg^{-1} $\cdot$ day^{-1} (the current RNI/RDA/DRI), 1.2 g protein $\cdot$ kg^{-1} $\cdot$ day^{-1} (at or close to the average protein intake for lacto-ovo, meat-eating male), or 2.2 g protein $\cdot$ kg^{-1} $\cdot$ day^{-1} (at or close to the habitual dietary protein intake for many strength training athletes).

Measures of muscle protein and whole-body protein kinetics, as well as nitrogen balance, should then be made at the beginning, in response to an acute bout of resistance exercise and at the end of their training programs, also in response to an acute bout of resistance exercise, which should last at least 12 weeks (Phillips 2002).

6 Protein supplements

Historically, strength athletes and weightlifters have always consumed a lot of protein (Katch et al. 1998; Marquart et al. 1998; Paul et al. 1998). A recent review of nutrient intakes of bodybuilders found that typical protein intakes were almost 200 g for competitive male bodybuilder (three times the recommended daily allowance [RDA] for protein) and 100 g for female bodybuilders (twice the RDA for protein) (Paul et al. 1998).

A survey of supplement use in high school athletes in 1984 found that 22% consumed protein drinks, 9% consumed amino acid supplements, and 17% consumed either weight-gain or weight-loss products (which contain high-levels of protein) (Marquart et al. 1998).

Commercial protein supplements may be convenient means for some busy athletes to secure additional protein in the diet. Many of these products contain high-quality protein, such as milk or egg protein; provide a balanced mixture of protein, carbohydrate and fat for additional calories; and may also contain supplemental vitamins and minerals (**Table 9**). *It is important to emphasize the point that these supplements should be used as an adjunct to an otherwise balanced nutritional plan, not as a substitute.*

Table 9. Protein Supplement Types

Protein powders (containing protein varying in purity from 34% to almost 100%)

Meal replacement powders (containing protein, carbohydrates, vitamins, minerals, and sometimes other nutrients or fats)

Weight gain powders (containing protein, carbohydrates, and sometimes fats, vitamins, minerals, and other nutrients)

Bars (some bars contain 25 g or more of protein and are advertised as a dietary protein source)

Ready-to-drink liquids (may or may not contain carbohydrates and other nutrients)

Pills (tablets, capsules, or wafers containing protein and sometimes other nutrients)

Protein hydrolysates (in powder, beverage, bar, or pill forms)

Amino acids (in combinations of 18 or more that approximate protein or mixtures of a few amino acids or individually – these may be stand-alone products or added to other protein-containing supplements)

Data from Bucci and Unlu 2000

7 Milk Proteins

7.1 Basic facts about milk proteins

Milk proteins (almost always bovine source) are commercially available as *1)* whole milk proteins, *2)* caseinates, and *3)* whey proteins (**Figure 20**). Milk protein is approximately 80% caseinate and 20% whey protein.

Figure 20. Milk-Based Protein Formulas. Recent advancements in extraction and purification of protein from various sources have made relatively pure proteins available at affordable prices. These advancements have removed most of the consumer objections to palability and organoleptic properties that labeled early protein supplements as foul tasting and unfriendly to gastrointestinal tracts. Most commercially available proteins are purified by similar means. Ultrafiltration of a solution containing protein, salts, carbohydrates, and fats through membranes with pore sizes larger than 0.1 µm retains fat micelles, leaving protein, carbohydrates and salts. Ultrafiltration through membranes with molecular weight cutoff of 5000 daltons retains proteins and removes salts, carbohydrates, and other small molecules. Diafiltration pumps distill water through the membrane to further remove small molecules, and increase protein concentration. Drying can be accomplished by a variety of methods, including evaporation and spray drying. For more detailed review *see* Bucci and Unlu 2000. Courtesy of Weider Nutrition International.

7.2 Whole milk proteins

Milk protein isolates are produced from skim milk by two basic methods: *1)* ultrafiltration, diafiltration (to concentrate protein) and drying, or *2)* low or high pasteurization, precipitation, washing, and drying. Milk protein concentrates and isolates contain more than 90% protein and are commonly used in foods and dietary supplements.

7.2.1 Effects of whole milk protein supplementation on exercise performance

Several Romanian studies involving a milk powder containing approximately 90 % protein and mineral salts (Ca, P, K, Na) have shown the ergogenic effects of this supplement. In one of these studies, 9 male and 8 female top Olympic athletes were given 1.2-1.5 g of milk protein $\cdot$ kg body mass^{-1} $\cdot$ day^{-1} during a period of 6 months (Dragan et al. 1985).

The milk protein was consumed in addition to 2.2-2.5 g protein $\cdot$ kg body mass^{-1} $\cdot$ d^{-1} in their diet. A control group with the same number of athletes and from the same sports was fed on the same diet, but without extra addition of milk protein. The effects of the addition of extra milk protein were monitored by estimating some parameters such as lean body mass, fat mass, muscle strength, protein and lipid composition in the blood serum, calcium metabolism, urinary mucoproteins, and liver and kidney functional tests. All athletes were under medical supervision during the experiment and no side effects were registered.

The results indicated that extra milk protein significantly improved physiological condition and led to better sports performance, even when compared to the controls. However, it is possible that these studies were confounded by interacting effects from exogenous anabolic agent use and/or by variable training/peaking preparations due to the timing of competitions held during the data collection.

Despite these experimental limitations, many strength athletes are convinced that these high-protein intakes are advantageous. If so, it should be possible not only to document this in a controlled setting, but also to clearly describe the underlying mechanism(s) responsible. This needs to be done before such high-protein intakes can be recommended (Lemon 2001). *Certainly, poorly controlled observations and testimonials from athletes are not acceptable substitutes for well-structured scientific studies.*

7.3 Whey proteins

7.3.1 Basic facts about whey proteins

Whey proteins are extracted from liquid whey produced as a byproduct of cheese or casein manufacturing (Bucci and Unlu 2000). Whey protein concentrates (~ 80 % protein) are produced from liquid whey by clarification, ultrafiltration, diafiltration, and drying. Whey protein isolates (>90% protein) are produced from liquid whey by a variety of techniques.

Whey proteins particularly have been singled out as the ultimate form of protein based on essential amino acid composition, branched-chain amino acid (BCAA) content, sulfur amino acid content, taste acceptance, ease of mixing, stability in liquids, and rapidity of digestion (Bucci and Unlu 2000).

7.3.2 Effects of whey protein supplementation on exercise performance, body composition and lymphocyte glutathione concentration

Lands et al. (1999) compared effects of whey protein concentrate (Immunocal, Immunotec Research, Vaudreuil, PQ) and casein on muscular performance, body composition and lymphocyte glutathione (GSH) concentration. GSH is a tripeptide that contains glutamic acid, cysteine, and glycine. GSH occurs widely in plant and animal tissues, and plays a major role in protecting skeletal muscle and other body tissues from oxidative damage.

In this study, twenty healthy young adults (10 men, 10 women) were studied and presupplementation and 3 months postsupplementation periods with either whey protein (20 g · day^{-1}) or casein placebo were analyzed. Exercise performance was assesses by whole leg isokinetic cycle testing, measuring peak power and 30-s work capacity. Lymphocyte GSH was used as a marker of tissues GSH. There were no baseline differences. Follow-up data on 18 subjects (9 whey protein, 9 placebo) were analyzed. Both peak power [13 ± 3.7 % (SE) %, $P < 0.02$] and 30-s work capacity (13 ± 3.7%, $P < 0.03$) increased significantly in the whey protein group. Lymphocyte GSH also increased significantly in the whey protein group, with no change in the placebo group (-0.9 ± 9.6%).

The exact mechanism(s) of how whey protein improved muscular performance is unclear. According to authors, the most obvious mechanism would be an increase in intracellular glutathione levels, leading to a decrease in oxidant-induced muscular dysfunction.

In addition, subjects on whey protein had a decrease in their percentage of body fat while maintaining their weight. Triceps and subscapular skinfold thicknesses were used to assess their percentage of body fat. According to Jones and Norgan (1998), skinfold thickness, when measured properly, correlates well ($r = 0.8$-0.9) with hydrostatic weighing, with a low standard error of estimate, that is 3 to 4 % of the body weight as fat.

7.4   Bovine colostrum

7.4.1   Basic facts about bovine colostrum

Bovine colostrums is the first milk secreted by cows after parturation and contains a number of bioactive proteins, including growth factors, of which insulin-like growth factor is one of the most abundant and well-characterized.

7.4.2 Bovine colostrum supplementation and IGF-I

7.4.2.1 Basic facts about IGF-I

Growth hormone (GH) is a protein anabolic hormone and produces positive nitrogen and phosphorous balance, and a fall in the blood urea nitrogen and amino acid levels. The effects of GH on growth, cartilage, and protein metabolism depend on an interaction between growth and somatomedins, which are polypeptide growth factors secreted by the liver and other tissues.

The principal (and in human probably the only) circulating somatomedins are insulin like growth factor I (IGF-I) and insulin-like growth factor II (IGF-II). IGF-II is though to be a less effective anabolic agent than IGF-I.

The majority of studies support the fact that IGF-1 has significant anabolic and anticatabolic effects, especially when acting with insulin and GH and where there is an adequate amount of certain amino acids (for review see Di Pasquale 1997). Thus, increasing endogenous levels of IGF-1 could be useful for maximizing the effects of exercise on muscle mass and strength.

7.4.2.2 Studies reporting increased IGF-I levels with bovine colostrum supplementation

The purpose of study by Mero et al. (1997) was to examine the effects of bovine colostrum supplementation (Bioenervi, Viable Bioproducts, Turku, Finland) on serum insulin-like growth factor I (IGF-I) concentrations during a strength and speed training period. Bovine colostrums supplement contained $67.6 \ \mu g \cdot l^{-1}$ IGF-I.

Nine male sprinters and jumpers underwent three randomized experimental training treatments of 8 days separated by 13 days. The only difference in the treatments was the drink of 125 ml consumed per day. Postexercise increases were noticed for serum IGF-I in the 25-ml bovine colostrum treatment (125 ml contained 25 ml BC) and especially in the 125-ml bovine colostrum treatment

(125 ml contained 125 ml BC) compared with the placebo (whey protein) treatment ($P < 0.05$). The change in IGF-I concentration during the 8-day periods correlated positively with the change in insulin concentration during the same periods with 25-ml BC treatment ($r = 0.68$; $P = 0.045$) and with 125-ml bovine colostrum treatment ($r = 0.69$; $P = 0.038$).

However, the investigators used a radioimmunoassay that measures both the IGF-I and its associated binding proteins. A more appropriate and accepted procedure is to remove the binding protein before measuring IGF-I (Owens et al. 1994).

Furthermore, the negligible change in IGF-I level could be due to the training effect. As might be expected and as in normal physiology, the observed exercise-associated rise in GH would be mirrored by a rise in circulating IGF-I. Cappon et al. (1994) reported a small (14%) rise in IGF-I within 10 min of the onset of high-intensity exercise that persisted for approximately 30 min and which was independent of GH release.

Finally, it should be noted that the initial mean level of IGF-I was somewhat greater in the placebo group. This means the possibility that reasons other than BC supplementation may have contributed to the differences in the IGF-I concentration between the groups.

In the follow-up study Mero et al. (2002) examined the effects of bovine colostrum (Dynamic Colostrum, Hi-Col Ltd, Finland) supplementation on blood and saliva variables (study 1) and the absorption of orally administered human recombinant IGF (rhIGF-1) labeled with [123]iodine (study 2).

In study 1 adult male and female athletes were randomly assigned in a double-blind fashion to either an experiment (bovine colostrum; $n = 19$) or a control (placebo; $n = 11$) group, the former consumed daily 20 g bovine colostrum supplement and the latter 20 g maltodextrin during a 2 week training

period. After bovine colostrum supplementation significant increases were noticed in serum IGF-1 ($P < 0.01$) in bovine colostrum compared with placebo.

In study 2 gel electrophoresis was carried out in 12 adult subjects with serum samples taken at 60 min after ingestion of ^{123}I-rhIGF-1 and showed peaks at 0.6-kDa and at 40 – 90-kDa, the former inducing 96% and the latter 4% of the total radioactivity.

Authors concluded that long-term supplementation of bovine colostrum increases serum IGF-1 concentration in athletes during training. Absorption data shows that ingested 123I-rhIGF-1 is fragmented in circulation and no radioactive IGF-1 is eluted at the positions of free or the IGF binding proteins giving no support to the absorption of intact IGF-1 from bovine colostrum.

The concentration of circulating IGF-1 increased in bovine colostrum with increasing usage time 14 days. The increase per day in the present study was slightly lower (0.38 nmol $\cdot$ l^{-1} $\cdot$ day^{-1}) than the respective value (0.54 nmol $\cdot$ l^{-1} $\cdot$ day^{-1}) in the earlier study by Mero et al. (1997) in which the supplementation time was 8 days.

7.4.3   Bovine colostrum supplementation and saliva IgA

7.4.3.1   Basic facts about IgA

Circulating antibodies protect their host by binding to and neutralizing some protein toxins, by blocking the attachment of some viruses to cells, by opsonizing bacteria and by activating complement. Antibody proteins are also known as immunoglubulins (Ig).

There are five Ig subclasses: IgG, IgA, IgM, IgD, and IgE. Most of the antibodies are in the serum are IgG subclass, whereas most of the antibodies in external secretions (saliva and milk) are IgA. Five general types of immunoglobulins are produced by the lymphocyte-plasma cell system.

The basic component of each is a symmetric unit containing four polypeptide chains. The two long chains are called heavy chains, whereas the two short chains are called light chains. The chains are joined by disulfide bridges that permit mobility, and there are intrachain bridges as well. Each heavy chain has variable (V) segment in which the amino acid sequence is highly variable, a diversity (D) segment in which the amino acid segment is also highly variable, a joining (J) segment in which it is moderately variable, and a constant (C) segment in which the sequence is constant.

In IgAs, the *secretory immunoglobulins*, the immunoglobulin units form dimers and trimers around a J chain and a polypeptide that comes from epithelian cells, the secretory component (SC).

In the intestine, bacterial and viral antigens are taken up by M cells and passed on to underlying aggregates of lymphoid tissue, where they stimulate lymphoblasts. These lymphoblasts then enter the circulation via the lymphatic ducts, but "after" maturation in the circulation, they move to diffuse lymphoid tissue underlying the intestinal mucosa and epithelial in the lungs, breast, genitourinary tract, and female reproductive tract. There they secrete large amounts of IgAs when exposed again to the antigen that initially stimulates them. The epithelian cells produce the SC, which acts as a receptor for and bind the IgA. The resulting secretory immunoglobulin passes through epithelial cell and is secreted by exocytosis. This system of secretory immunity is an important and effective defense mechanism.

Because the relatively high incidence of upper respiratory track infection (URTI) symptoms among endurance athletes and the importance of mucosal IgA to resistance to viral infections, several researcher groups have focused on the IgA response to exercise. Salivary IgA concentrations have been shown to decline acutely after intense exercise (for review see Mackinnon 1999). Resting salivary IgA concentration is either normal or low in competitive athletes compared with nonathletes.

Two studies suggest a relationship between declining salivary IgA concentration and the appearance of URTI in elite athletes (Mackinnon et al. 1993; Gleeson et al. 1999). In one study of elite squash and field hockey athletes, declines in salivary IgA during acute intense exercise predicted the appearance of URTI within the following 2 d (Mackinnon et al. 1993). In the other study, low resting and post-exercise IgA levels were predictive of development of URTI over a season in elite swimmers. (Gleeson et al. 1999).

To date, salivary IgA concentration is the only immune parameter to be correlated with the appearance of URTI in athletes. Interestingly, IgA output and the number of IgA producing cells are normal or slightly elevated in duodenum biopsies from marathon runners compared with nonathletes (Nilssen et al. 1998), suggesting that the effects of intense exercise training may be specific to the respiratory tract.

7.4.3.2 Effects of bovine colostrums supplementation on salivary IgA concentrations

A novel finding by Mero et al. (2002) was the 33% increase in salivary IgA concentrations during two weeks of bovine colostrum supplementation. In the earlier study by Mero et al. (1997) there was no change in salivary IgA during 8 days. In the present study the daily amount of IgA was 0.3 g, which is greater than in the earlier study. This may be the main reason for the increase of salivary IgA.

7.4.4 Effects of bovine colostrum supplementation on exercise performance and body composition

The purpose of recent study by Antonio et al. (2001) was to determine the effect of 8 wk of bovine colostrum supplementation on body composition and exercise performance in active men and women.

Subjects were randomly assigned to a placebo (whey protein) and bovine colostrum group (20 g · day^{-1} in powder form). Each subject participated in aerobic and heavy-resistance training at least three times per wk. Body composition was assessed via dual x-ray absorptiometry (DEXA) analysis. Treadmill time to exhaustion, one repetition maximum strength (bench press), and the total number of repetitions performed during one set to exhaustion at a submaximal load for the bench press (50% and 100% of body weight for women and men, respectively) were ascertained.

The whey protein group experienced a significant increase ($P < 0.05$) in body weight (mean increase of 2.11 kg), whereas the bovine colostrum group experienced a significant ($P < 0.05$) increase in bone-free lean body mass (LBM) (mean increase of 1.49 kg). There were no changes in any of the other parameters measured. The coefficient of variation for estimated LBM using DEXA is approximately 1% (unpublished observations from authors laboratory). This would be equal to a 0.64 kg change in LBM. Thus, it might be surmised that the 1.5-kg increase in LBM in the bovine colostrum-supplemented group may in fact be a true increase.

However, one must be cautious with this interpretation because other investigators have found the coefficient of variation for LBM estimates via DEXA to be as high as 3.1%. Certainly, this is as large as the difference in LBM reported for the bovine colostrum-supplemented group.

Even though LBM increased, body weight did not change significantly. According to authors, this may have been owing to the slight decrease in body fat mass. It is not known what underlying mechanism(s) is responsible for the purported gains in LBM as a result of bovine colotrum supplementation.

The purpose of the recent study by Coombes et al. (2002) was to determine the dose effects of bovine colostrum on cycling performance. Forty-two competitive cyclists were randomly divided into three groups and required to

consume either 20 g · day^{-1} bovine colostrum + 40 g whey protein, 60 g of bovine colostrum, or 60 g of whey protein (placebo).

Two measures were used to assess performance before (pre-) and after (post-) an 8-wk supplementation period. The first measure required subjects to complete two VO_{2max} tests separated by 20 min with the amount of work completed in the second test used to evaluate performance. The second performance measure was the time to complete a work-based time trial following a 2-h cycle at 65% VO_{2max}. Subjects were required to maintain their regular training and keep a food and training diary over the study period.

After supplementation, the performance enhancement in Measure One was not statistically significantly different in the colostrum groups compared to the placebo group (placebo = 3.4%, 20 g = 4.0%, 60 g = 3.9%; 95% confidence interval (CI) for differences, ± 1.8%, $P > 0.05$).

In performance Measure Two subjects in the 20 g and 60 g groups completed the time trial significantly ($P < 0.05$) faster post supplement compared to pre supplement (improvements in performance times, placebo = 37 s, 20 g = 158 s, 60 g = 134 s; 95% CI for differences, 47 s).

Authors concluded that oral bovine colostrum supplementation at 20 g or 60 g · day^{-1} provided a small but significant improvement in time trial performance in cyclists after a 2-h ride at 65% VO_{2max}.

Further, authors postulated that bovine colostrums supplementation improves small intestine function and nutrient absorption leading to enhanced nutrient availability to the recovering muscle cells. According to authors, it is well established that in adult animals administration of IGF-I increases intestinal mucosal weight, protein and DNA content, villus height, and epithelial proliferation and function.

8 Soy protein

8.1 Basic facts about soy proteins

Soy protein has been a subject for extensive industrial research to produce a virtually pure protein isolate in commercial quantities (Bucci and Unlu 2000). Soy protein is produced from soybeans via water extraction, followed by precipation, washing and drying procedures to yield either a soy protein concentrate (~70% protein) or soy protein isolates (~90% protein). Some extraction procedures use ethanol/water mixtures, which remove isoflavones (Bucci and Unlu 2000).

In the past, low protein purity of soy-derived protein sources led to consumer dissatisfaction due to gastrointestinal disturbances, poor taste, and poor mixibility. Although these objections have been successfully eliminated, the image of poor tolerance of soy protein remains in the minds of many consumers. The significantly lower cost of soy protein isolates compared to egg, whey, and some milk proteins have made soy protein supplements more attractive.

8.2 Isoflavone phytoestrogens

8.2.1 Basic facts about estrogen

Estrogen, in addition to its paracrine function within the ovaries, its effects on the anterior pituitary and the hypothalamus, and its uterine actions, exerts a large number of other effects, as summarized in **Table 10**.

Table 10. Effects of Estrogens

1. Stimulates growth of ovary and follicles.

2. Stimulates growth of smooth muscle and proliferation of epithelial linings of reproductive tract.

3. Stimulates external genitalia growth.

4. Stimulates breast growth, particularly ducts and fat deposition.

5. Stimulates female body configuration development.

6. Stimulates a more-fluid sebaceous gland secretion.

7. Stimulates development of female pubic hair pattern.

8. Stimulates bone growth and ultimate cessation of bone growth

9. Vascular effects (deficiency produces "hot flashes")

10. Has feedback effect on hypothalamus and anterior pituitary

11. Stimulates fluid retention by kidneys.

12. Stimulates prolactin secretion but inhibits prolactin´s milk-inducing action on the breasts.

13. Protects against atherosclerosis by effects on plasma cholesterol.

14. Exerts effects on brain neurons that may enhance learning and memory.

Modified from Vander et al. 2001.

The cause of bone loss after menopause is primarily estrogen deficiency. Estrogens inhibit secretion of cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor α, and these cytokines foster the development of osteoclasts (Ganong 2001c). Estrogens also stimulate production of transforming growth factor β, and this cytokine increases apoptosis of osteoclasts (Ganong 2001c). There are estrogen receptors on osteoblasts, and a direct stimulatory effect on them is a possibility.

Furthermore, estrogens have a significant plasma cholesterol-lowering action and they rapidly produce vasodilation by increasing the local production of nitric oxide (Ganong 2001d). These actions inhibit atherogenesis and contribute to the low incidence of myocardial and other complications of atherosclerotic vascular disease in premenopausal women.

Like other steroids, estrogens combine with protein receptors in the nucleus, and the complex binds to DNA, promoting formation of the new proteins which modify cell function. Two estrogen receptors have been cloned: estrogen receptor α (ER α) and estrogen receptor β (ER β).

Although there is overlap, the distribution of these receptors is different. ER α expression is moderate to high in uterus, testis, pituitary, kidney, epididymis, and adrenal, whereas ER β expression is high in the ovary, prostate, lung, bladder, and bone (Ganong 2001d). Male and female mice in which the gene for ER α has been knocked out are sterile, develop osteoporosis, and continue to grow because their epiphyses do not close (Ganong 2001d).

8.2.2 Isoflavone phytoestrogens

Phytoestrogens are ubiquitous, nonsteroidal, plant-derived compounds reported to exhibit both estrogen agonist and antagonist activities (Teede et al. 2001). The soybean is a rich source of the isoflavone phytoestrogens genistein and daidzein, ligands for both estrogen receptors ER α and ER β, with a greater affinity demonstrated for ER β (Teede et al. 2001).

8.2.2.1   Phytoestrogens and bone health

Isoflavones have been studied in postmenopausal osteoporosis and generally found to have positive effect in maintaining bone density and reducing fractures (Ilich and Kerstetter 2000). However, since they are relatively new agents, the long-term benefits or consequences, as well as their impact on fracture reduction, need more clarification.

8.2.2.2   Phytoestrogens and serum lipid profile

The most consistently demonstrated beneficial effect of soy has been on lipids (Anderson et al. 1995). Federal Drug Administration approval for consumer labeling of soy foods has given in the United States, stating that "*included in the daily diet, they may reduce the risk of heart disease*".

This is based primarily on the lipid benefits. A meta-analysis of 38 published controlled human clinical trials, of an average of 47 g daily soy protein consumption (primarily in subjects with hyperlipidemia), noted significant reduction in total and LDL cholesterol and triglycerides, with the hypocholesterolemic effect significantly related to pretreatment cholesterol levels (Anderson et al. 1995).

8.2.2.3 The threshold intake of isoflavones in humans to achieve biological activity

The threshold intake of dietary isoflavones in humans to achieve biological activity is thought to be $30 - 50$ mg $\cdot$ day^{-1} (Setchell 1998). Intakes in the 30 to 50 mg $\cdot$ day^{-1} range may influence serum lipids, but higher doses may be required to influence bones (Ilich and Kerstetter 2000).

For example, moderate amounts of isoflavones (56 mg $\cdot$ day^{-1}) did not affect bone mineral density (BMD) over a six-month period; however, a 90 mg $\cdot$ day^{-1} dose (taken as a supplement) was needed to show increase in BMD of the lumbar spine in a group of postmenopausal women (Potter et al. 1998).

8.2.2.4 Amenorrhea and soy protein

Amenorrhea is the absence of the menstrual periods. Cessation of cycles in a woman with previously normal periods is called secondary amenorrhea. The entity of "athletic amenorrehea" has been described recently. Amenorrhea itself occurs in approximately 5% of the general population, while it may be present in up to 20% of women who exercise, and up to 50% of elite athletes (Highet 1989).

Amenorrhea (due to suppressed estrogen levels) can cause osteoporosis. Osteoporosis is the loss of bone minerals. It occurs in postmenopausal women. Numerous studies have shown that young amenorrheic women athletes experience decreased bone density and fail to achieve their predicted peak bone density.

Athletic amenorrhea may also increase the risk of coronary heart disease. Estrogens increase the blood levels of high-density lipoproteins and apoprotein A-1 (though to provide some protection against coronary heart disease) and decreases low-density lipoproteins.

Drinkwater et al. (1990) found that estradiol levels in amenorrheic runners were less than half of normally menstruating runners. Thus, I would hypothesize that soy protein isolate is ideal protein source for young amenorrheic athletic women. This hypothesis needs to be examined.

8.3   Soy protein isolate and amino acid profile

Soy protein contains a higher percentage (35%) of five "critical cluster" amino acids, including glutamine, arginine, and the branched chain amino acids (BCAA) than other proteins, such as whey, casein, egg, and beef (**Figure 21**). In theory, these are the amino acids preferred for fortification of proteins by the dietary supplement industry, and they have hypothetical benefits for exercising individuals.
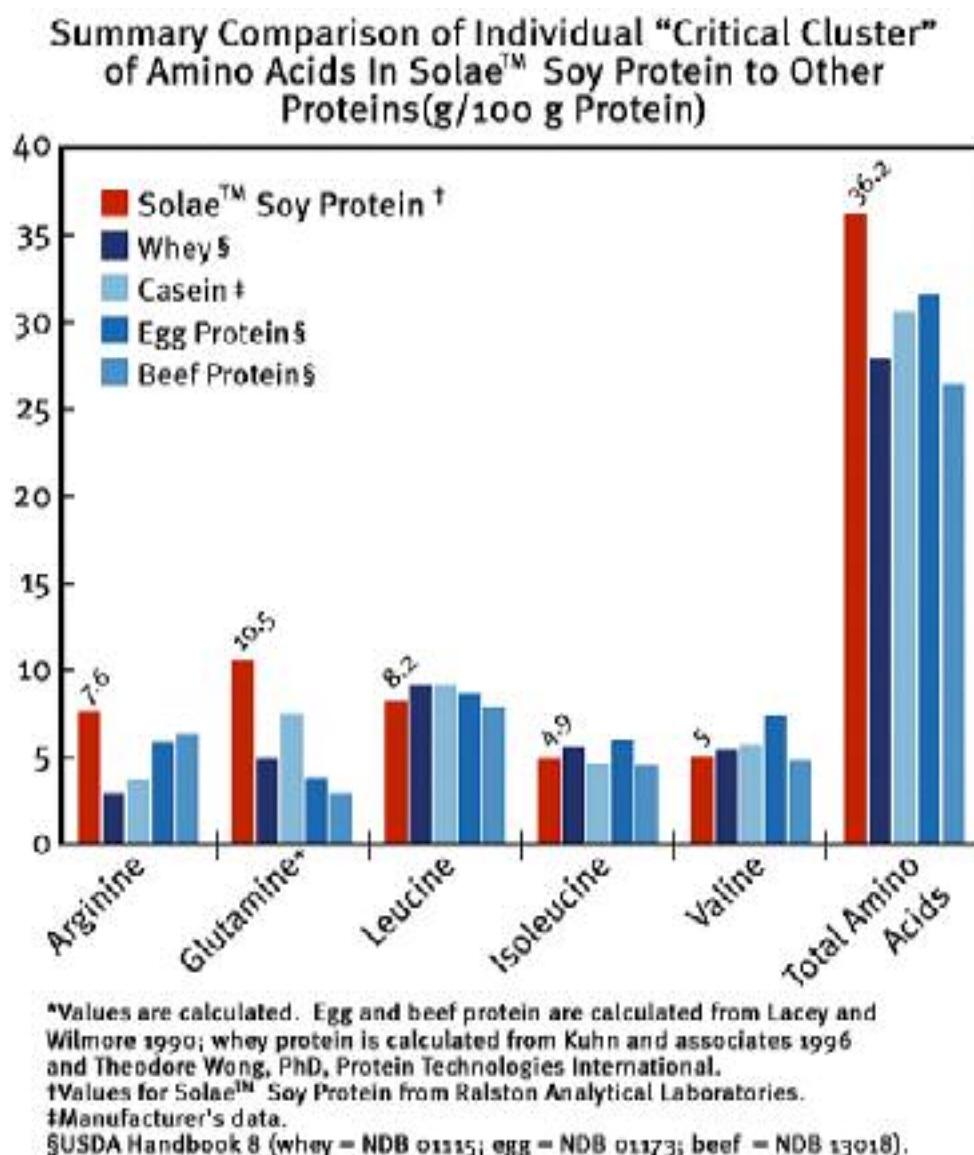
Figure 21. **Diagram Illustrating the Amount of "Critical Cluster" Amino Acids in Different Protein Sources.** These amino acids are hypothesized to be of primary importance, among amino acids, for muscle metabolism. Solae™ refers to isolated soy protein. *Data from* Protein Technologies International.

Soy protein isolates are generally less expensive than whey and egg proteins, and possess favorable organoleptic properties such as mixibility, taste, texture, and ease of flavoring. In addition, recent data has confirmed that soy protein is complete for humans, and has similar biological value, as illustrated by PDCAAS score, in humans to milk, beef, and egg proteins (**Figure 18**).

This is in spite of fact that soy protein-based formulas have been used successfully for many years in growth of infants as the sole source of protein. Infant growing have the highest requirements of sedentary humans, and thus represent a practical test for biological value of soy protein in humans.

8.4 Studies of soy protein supplementation in exercising humans

Four double-blind, placebo-controlled studies on elite Romanian athletes administered $1.5 \text{ g} \cdot \text{kg body mass}^{-1} \cdot \text{d}^{-1}$ additional protein from isolated soy protein for 8 to 16 weeks during stressful training periods. (Dragan et al. 1992, 1993; Stroescu 1994,1996).

Athletes ranged from endurance sports to swimmers to gymnasts. In general, lean body mass was preserved or increased, muscular strength was maintained or increased, and urinary mucoproteins were decreased in soy protein-supplemented subjects, while adverse effects were noticed in placebo subjects.

However, it is possible that these studies were confounded by interacting effects from exogenous anabolic agent use and/or by variable training/peaking preparations due to the timing of competitions held during the data collection.

Moreover, it should be noted that these studies have not been published in major peer-reviewed journals. To my knowledge, only one study on soy protein supplementation and exercise has been published in major peer-reviewed journal.

Stroescu et al. (2001) evaluated the metabolic and hormonal response in elite female gymnasts undergoing strenuous training and supplementation with isolated soy protein (SUPRO, Protein Technologies International).

Top female gymnasts (n = 14) took part in this study to examine their hormonal metabolic profile and to investigate any possible changes resulting from a 4-months program of strenuous training and daily supplementation with soy protein at a level of 1 g · kg body mass^{-1}. Gymnasts were randomly assigned to one of two groups: seven to the supplemented group (A) and seven to the non-supplemented group (B). Both groups took part in the same program, which consisted of strenuous training for 4-6 h · day^{-1} (except on Sunday). Group A received a supplement with isolated soy protein twice daily. Group B received a placebo identical in appearance and flavor.

Selected parameters were measured before and after the 4-month training program: lean body mass, fat mass, serum hemoglobin, protein, fats, urea and creatinine, liver enzymes, serum total calcium and magnesium, immunoglobulins, urinary mucoproteins, serum T_3 and T_4, estradiol, progesterone, prolactin, testosterone, and urinary 17-ketosteroids.

Results demonstrated that the supplemented group had an increase in lean body mass and serum levels of prolactin ($P < 0.01$) and T_4 and a decrease in serum alkaline phosphatases ($P < 0.01$). The non-supplemented group had a decreased level of serum T_4 and an increased level of urinary mucoproteins ($P < 0.05$).

According to authors, these results might suggest lower metabolic-hormonal stress in elite female gymnasts undergoing strenuous training and who received daily supplementation with isolated soy protein.

Although more research is needed, soy protein is an under-recognized means of providing high-quality protein for exercising individuals and perhaps may possess other benefits due to isoflavone content and actions.

9 Protein hydrolysates

Protein hydrolysates are produced from purified protein sources by heating with acid or preferably, addition of proteolytic enzymes, followed by purification procedures as outlined for proteins, with lower molecular weight cutoff for membrane filtration steps. Enzyme hydrolysis is greatly preferred because acid hydrolysis oxidizes cysteine and methionine, destroys some serine and threonine, and converts glutamine and asparagine to glutamate and aspartate, respectively, lowering protein quality and biological value (Bucci and Unlu 2000).

9.1 Protein digestion and absorption

Protein digestion begins in the stomach, where pepsins cleave some of the peptide linkages. Pepsins hydrolyze bonds between aromatic amino acids such as phenylalanine or tyrosine and a second amino acid, so the products of peptic digestion are polypeptides of very diverse sizes. In the small intestine, the polypeptides formed by digestion in the stomach are further digested by the powerful proteolytic enzymes of the pancreas and intestinal mucosa.

At least seven different transport systems transport amino acids into enterocytes (Ganong 2001a). Five of these require Na^+ and cotransport amino acids and Na^+ in a fashion similar to the cotransport of Na^+ and glucose. Two of these four also require Cl^-. In two systems, transport is independent of Na^+. Di- and tripeptides are transported into enterocytes by a system that requires H^+ instead of Na^+ (Ganong 2001a). There is very little absorption of larger peptides.

9.2 Advantages of protein hydrolysates

Several studies have shown that protein hydrolysates made up mostly of di- and tripeptides are absorbed more rapidly than amino acids and protein hydrolysates containing larger peptides, and much more rapidly than whole foods (Grimble and Silk 1986; Grimble et al. 1987).

The considerably greater absorption rate of amino acids from the dipeptide than from the amino acid mixture appears to be the result of uptake by a system that has a greater transport capacity than amino acid carrier system, thus minimizing competition among its substrates (Steinhardt and Adibi 1986). This is a desirable trait for athletes who wish to maximize amino acid delivery to muscle.

Furthermore, in a study by Boza et al. (1994) it was shown that whey protein hydrolysate has a significantly higher BV than the whole protein. This corroborates an earlier study that found a higher nitrogen retention balance in rats fed a diet containing whey protein hydrolysate compared with native protein (Poullain et al. 1989).

However, whether this apparent advantage over ingestion of foodstuffs has a practical effect of faster muscle mass accretion or improved recovery from exercise has not been adequately studied in exercising individuals (Bucci and Unlu 2000). Nevertheless, documented advantages (faster uptake of amino acids, higher BV) remain attractive to consumers.

Furthermore, milk-based protein hydrolysates may be superior to whole foods for use in people who have certain food allergies. Hypoallergenic protein hydrolysates are prepared in such a way that the quantity of antigenic material is drastically reduced and thus can be used by those who are normally allergic to milk proteins. The enzymatic hydrolysis of protein coupled with ultrafiltration is the best way to reduce their antigenicity, and also preserving the nutritional qualities of the amino acids and the peptides produced (Di Pasquale 1997).

9.3 Protein hydrolysates and insulin secretion

9.3.1 Basic facts about insulin

The physiological effects of insulin are far-reaching and complex. They are conveniently divided into rapid, intermediate, and delayed actions, as listed in **Table 11**. The best know is the hypoglycemic effect, but there are additional effects on amino acid and electrolyte transport, many enzymes, and growth. The net effect of the hormone is storage of carbohydrate, protein, and fat.

Table 11. Principal Actions of Insulin

Rapid (seconds)
Increased transport of glucose, amino acids[a], and K^+ into insulin-sensitive cells
Intermediate (minutes)
Stimulation of protein synthesis[b]
Inhibition of protein degradation
Activation of glycolytic enzymes and glycogen synthase
Delayed (hours)
Increase in mRNAs for lipogenic and other enzymes

Data from Ganong 2001b
[a]Among the amino acids most strongly transported are valine, leucine, isoleucine, tyrosine and phenylalanine (Guyton and Hall 2000).
[b]Insulin increases the translation of mRNA, thus forming new proteins. In some unexplained way, insulin "turns on" the ribosomal machinery.

Formerly, it was believed that insulin secretion was controlled almost entirely by the blood glucose concentration. However, as more has been learned about the metabolic functions of insulin for protein and lipid metabolism, it has become apparent that blood amino acids and other factors also play important roles in controlling insulin secretion.

Amino acids administered in the absence of a rise in blood glucose cause only a small increase in insulin secretion. However, when administered at the same time that blood glucose concentration is elevated, the glucose-induced secretion of insulin may be as much as doubled in the presence of the excess amino acids (Guyton and Hall 2000).

9.3.2 Effects of protein hydrolysates and free amino acids on insulin secretion

In recent study by van Loon et al. (2000c), a total of 10 drinks were tested in 8 nonobese males after an overnight fast to investigate the insulinotropic potential of several free amino acids, protein hydrolysates, and an intact protein.

At 0, 30, 60, and 90 min, the subjects received a beverage 3.5 mL/kg to ensure a given dose of 0.8 g carbohydrate $\cdot$ kg^{-1} (50% as glucose and 50% as maltodextrin) and 0.4 g $\cdot$ kg^{-1} of an amino acid and protein hydrolysate mixture every hour. The results of this study indicate that oral ingestion of some amino acid mixtures in combination with carbohydrates can produce strong insulinotropic effects.

To compare the insulinotropic effect of the ingestion of the protein hydrolysates with that of an intact protein, sodium-caseinate was provided in one of the drinks. This resulted in an insulin response that was not significantly different from that found with the control trial (30% greater) and tended to be less than the responses observed after ingestion of the protein hydrolysates (**Figure 22**). After ingestion of the intact protein, plasma amino acid responses over this 2-h period were in general lower than the responses observed after ingestion of the protein hydrolysates.
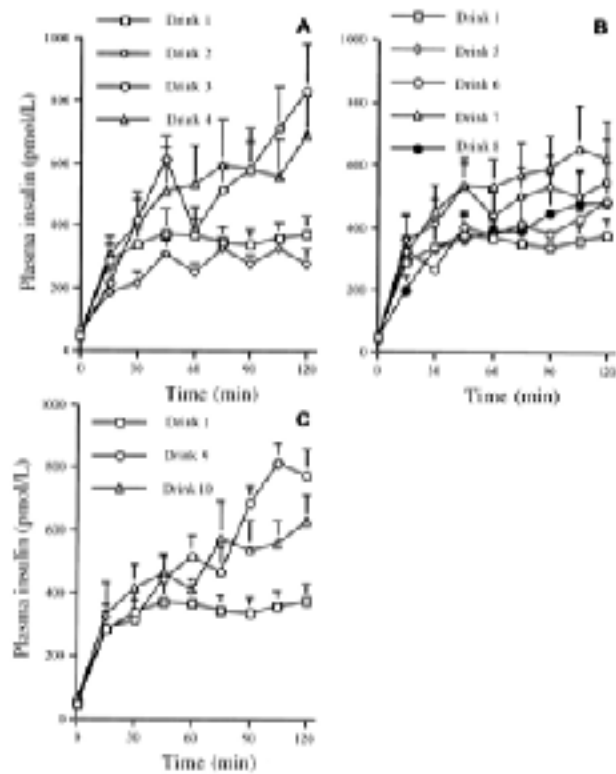
Figure 22. Mean (±) Plasma Insulin Concentration After Ingestion of the Control Drink and Drinks Containing Hydrolysates and an Intact Protein (B), or the Control Drink and Drinks Containing Mixtures of Hydrolysates and Free Amino Acids (C). *n* = 8. Drink 1 = carbohydrates only (glucose + maltodextrin); Drink 2 = arginine + carbohydrates; Drink 3 = arginine + leucine + phenylalanine + carbohydrates; Drink 4 = arginine + glutamine + leucine + phenylalanine + carbohydrates; Drink 5 = whey hydrolysate + carbohydrates; Drink 6 = pea hydrolysate + carbohydrates; Drink 7 = wheat hydrolysate + carbohydrates; Drink 8 = casein + carbohydrates; Drink 9 = leucine + phenylalanine + wheat hydrolysate + carbohydrates; Drink 10 = arginine + leucine + phenylalanine + wheat hydrolysate. For the exact composition of the different drinks, *see* van Loon et al. 2000c.

Regression analysis of the insulin responses and the changes in the plasma amino acids concentrations over the 2-h period showed a strong positive correlation between the observed insulin response and changes in plasma leucine ($P < 0.003$), phenylalanine ($P < 0.0001$), and tyrosine ($P < 0.0001$) concentrations.

This agrees with several *in vivo* studies in which β-cells of pancreas were incubated with leucine and phenyalanine and with the *in vivo* studies by Floyd et al. in which amino acids were infused (for references see van Loon et al. 2000c). The correlation observed with tyrosine concentrations may be

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explained by the fact that tyrosine is formed by the hydroxylation of phenylalanine when large amounts of phenyalanine are ingested.

In addition, investigators observed an unexplained positive correlation with citrulline ($P < 0.002$) and a negative correlation with glutamine ($P < 0.019$). The addition of free glutamine hardly influenced plasma glutamine levels. The data in this study show clearly that oral ingestion of large amounts of free arginine is not an effective means of increasing plasma insulin concentrations and plasma arginine concentrations.

The main conclusion is that oral intake of protein hydrolysates and amino acids in combination with carbohydrates can result in an insulinotropic effect as much as 100% greater than with the intake of carbohydrates only.

In another study by van Loon et al. (2000a), after an overnight fast, eight male cyclists visited at laboratory on five occasions, during which a control and two different beverage compositions in two different doses were tested.

After they performed a glycogen-depletion protocol, subjects received a beverage (3.5 mL $\cdot$ kg^{-1}) every 30 min ensure an intake of 1.2 g $\cdot$ kg^{-1} $\cdot$ h^{-1} carbohydrate and 0, 0.2 or 0.4 g $\cdot$ kg^{-1} $\cdot$ h^{-1} protein hydrolysate and amino acid mixture.

After the insulin response was expressed as the area under curve, only the ingestion of the beverages containing protein hydrolysate, leucine and phenylalanine resulted in a marked increase in insulin response compared with carbohydrate-only trial (**Figure 23**). A dose-related effect existed because doubling the dose (0.2-0.4 g $\cdot$ kg^{-1} $\cdot$ h^{-1}) led to an additional rise in insulin response ($P < 0.05$). Plasma leucine, phenylalanine and tyrosine concentrations showed strong correlations with the insulin response ($P < 0.0001$).
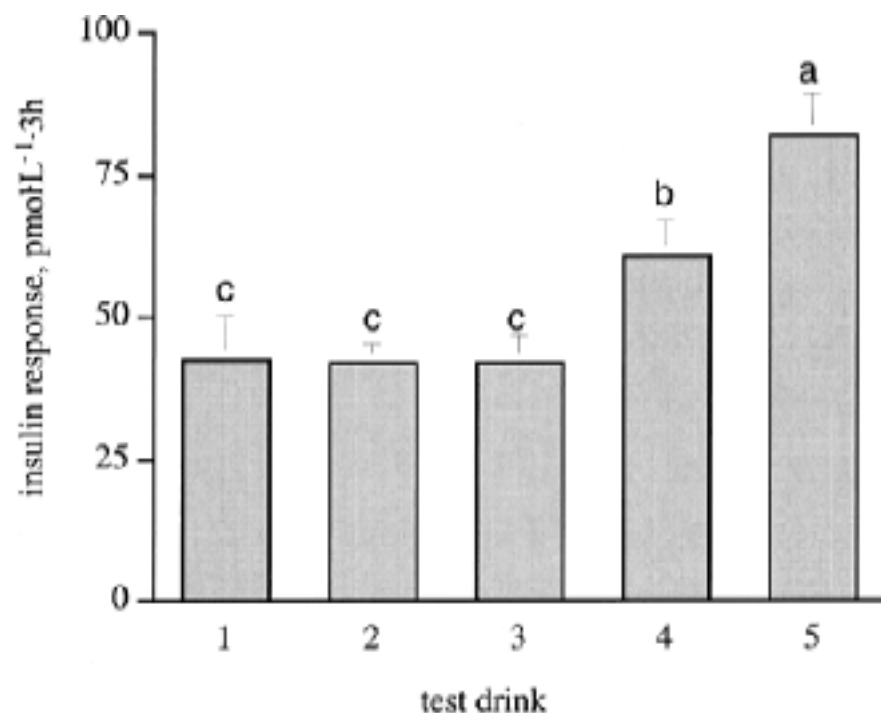
Figure 23. **Post-Exercise Plasma Insulin Responses After the Ingestion of Protein Hydrolysate/Amino Acid-Carbohydrate Mixtures in Humans.** Drink 1 = carbohydrate only (40:60% maltodextrin/glucose, 1.2 g · kg^{-1} · h^{-1}); Drink 2 = carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}); Drink 3 = carbohydrate with protein hydrolysate (0.4 g · kg^{-1} · h^{-1}); Drink 4 = carbohydrate with protein hydrolysate (0.1 g · kg^{-1} · h^{-1}), leucine (0.05 g · kg^{-1} · h^{-1}) and phenylalanine (0.05 g · kg^{-1} · h^{-1}); Drink 5 = carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}), leucine (0.1 g · kg^{-1} · h^{-1}) and phenylalanine (0.1 g · kg^{-1} · h^{-1}). Values are means ± SEM ($n = 8$). Mean values not sharing a common superscript are different, $P < 0.05$. The protein hydrolysate is prepared from wheat protein via enzymatic digestion and has a medium chain length of 11 amino acids. *Data from* van Loon 2000a.

Plasma amino acid concentrations were generally lower after the ingestion of drinks 4 and 5 compared to with the control drinks, although in the latter, considerable amount of protein and amino acids were ingested. This seems to suggest that tissue amino acid uptake and possibly also post-exercise net muscle protein balance were increased after the ingestion of this insulintropic mixture.

This would be in line with several studies demonstrating that an increase in plasma insulin concentration, during conditions of hyperaminoacidemia, further increases net muscle protein balance in vivo in humans (for references see van Loon et al. 2000a.). Such a stimulating effect on net protein balance may in part also be a consequence of a stimulating effect of leucine on skeletal

muscle protein synthesis, independent of an increase in insulin levels (Anthony et al. 1999, 2000).

According to authors, "***This study provided a practical tool to markedly elevate insulin levels and plasma amino acid availability through dietary manipulation, which may be of great value in clinical nutrition, (recovery) sports drinks and metabolic research***."

However, the potential of insulintropic protein hydrolysate and amino acid mixtures to stimulate post-exercise net muscle protein anabolism, and the mechanisms involved, remains to be investigated.

9.4 Protein hydrolysates and post-exercise glycogen synthesis

9.4.1 Basic facts about glycogen synthesis

After absorption into a cell, glucose can be used immediately for the release of energy to the cells, or it can be stored in the form of glycogen, which is a large polymer of glucose. In well-nourished humans, approximately 375 to 475 g of carbohydrate is stored in the body. Of this, approximately 325 g is muscle glycogen (largest reserve), 90 to 110 g is liver glycogen (highest concentration that represent between 3 to 7% of the liver´s weight), and only approximately 5 g is present as blood glucose (McArdle et al. 2001).

The formation of glycogen from glucose is called glycogenesis. In this process, glucose is converted to glucose 6-phosphate by utilizing the terminal phosphate group of ATP. Glucose 6-phosphate is then converted into its isomer, glucose 1-phosphate. Finally, the enzyme *glycogen synthase* removes these phosphate groups as it polymerises glucose to form glycogen (**Figure 24**).
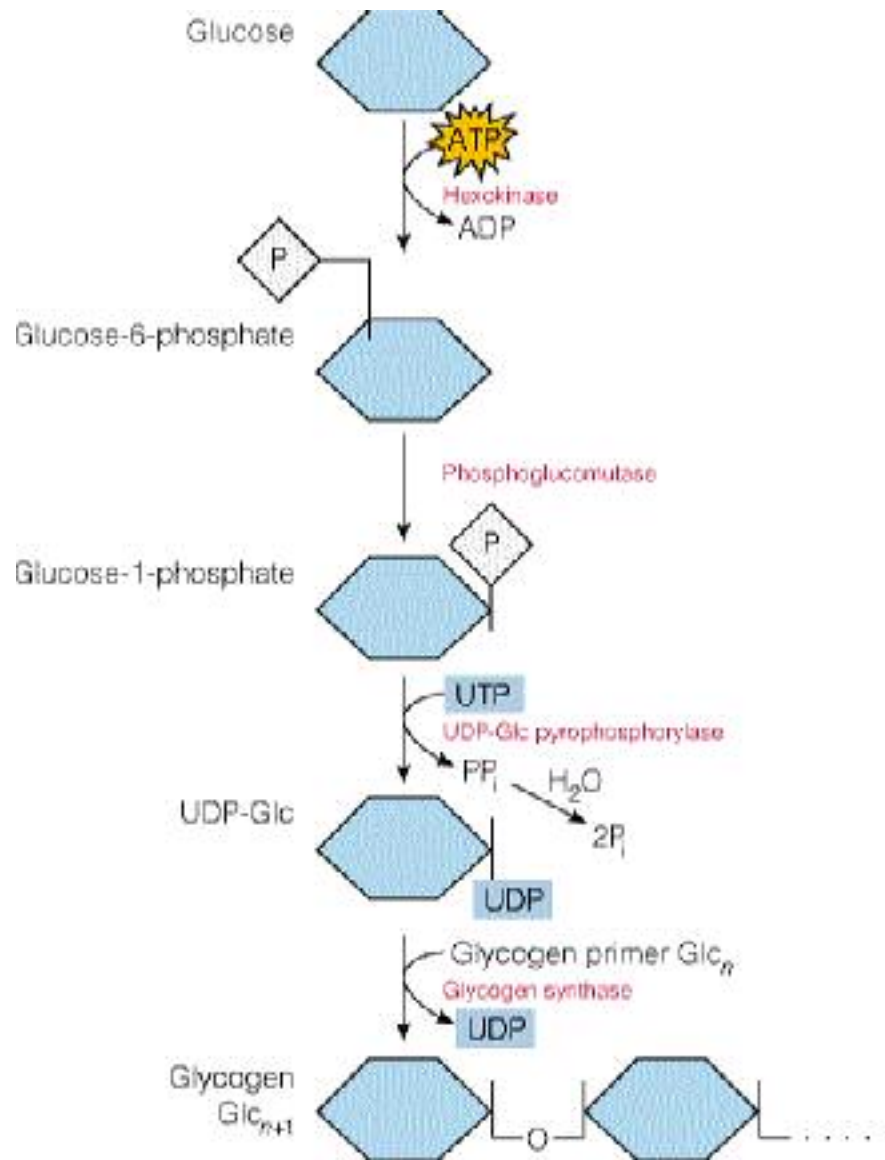
Figure 24. **Pathways for Conversion of Glucose Monomers to Polymeric Glycogen.**
Stage 1. ATP donates a phosphate to glucose to form glucose 6-phophate. This reaction involves the enzyme hexokinase (muscle) or glucokinase (liver). *Stage 2.* The enzyme phosphoglucomutase catalyses the isomerization of glucose 6-phophate to glucose 1-phosphate. *Stage 3.* The enzyme uridyl transferase reacts with glucose 1-phosphate to form UPD-glucose (a pyrophosphate forms in the degradation of uridine triphosphate). *Stage 4.* UDP-glucose attaches to one end of an already existing glycogen polymer chain. This forms a new bond (known as a glycoside bond) between the adjacent glucose units, with concomitant release of UDP. For each glucose unit added, two molecules of high-energy phosphate (ATP and UDP) convert to two molecules of ADP and inorganic phosphate. Glycogen synthase exists in two forms. Glycogen synthase I (GS I) is the unphosphorylated form that is normally active. Glycogen synthase D (GS D) is the phosphorylated form that is normally inactive but can become active if the glucose 6-P concentration increases. Phosphorylation inactivates the active form of glycogen synthase (GS I). A variety of kinases are capable of phosphorylating GS I, including phopshorylase kinase, cAMP-dependent protein kinase, and a calcium-dependent protein kinase. Conversion of inactive GS D to active GS I occurs by dephosphorylation, catalysed by protein phosphatase-1. *Insulin enhances the effect of protein phosphatase-1, stimulating the formation of GS I to help make glycogen.* Epinephrine increases the concentration cAMP, leading to phosphorylation and hence inactivation of glycogen synthase in muscle. *Reproduced from* Mathews et al. 2000.

Fatigue during prolonged exercise is often associated with muscle glycogen depletion; therefore, high pre-exercise muscle glycogen concentrations are believed to be essential for optimal performance. Because endurance athletes often train twice daily for several days and may compete on consecutive days, rapid restoration of muscle glycogen is of crucial importance to optimize recovery.

9.4.2   Post-exercise glycogen synthesis

The complete restoration of muscle glycogen after prolonged exercise can occur within 24 h, depending on the degree of glycogen depletion and provided that sufficient carbohydrates (CHO) are ingested. Consuming food after exercise facilitates glucose transport into muscle cells by:

- Enhanced hormonal milieu, particularly higher insulin and lower catecholamine levels.

- Increased sensitivity to insulin and intracellular glucose transporter proteins.

- Increased activity of glycogen synthase.

Consuming high-glycemic, carbohydrate-rich foods as soon as possible after hard training or competition speeds glycogen resynthesis. With optimal carbohydrate intake, glycogen stores replenish at approximately 5 to 7% per hour. Thus, under the best circumstances, it takes at least 20 hours to reestablish glycogen stores after a glycogen-depleting bout of exercise.

It has been suggested that muscle glycogen synthesis after glycogen-depleting exercise occurs in two phases (Price et al. 1994). Initially, there is a period of rapid synthesis of muscle glycogen that does not require the presence of insulin and lasts approximately 30-60 min (Jentjens et al. 2001). This early post-exercise recovery period is marked by an exercise-induced permeability of the muscle cell membrane to glucose (Jentjens et al. 2001).

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The glucose transporters (GLUT) that are responsible for facilitated diffusion of glucose across cell membrane are a family of closely related proteins that cross the cell membrane 12 times and have their amino and carboxyl terminals inside the cell. GLUT 4 is the transporter in muscle and adipose tissue that is stimulated by insulin (Ganong 2001b). A pool of GLUT 4 molecules is maintained in vesicles in the cytoplasm of insulin-sensitive cells. When the insulin receptors are activated, the vesicles move rapidly to the cell membrane.

GLUT 4 translocation occurs during exercise, and the increase in the density of GLUT 4 transporters in the muscle membrane seems to persist for some time after exercise (Jentjens et al. 2001). The second phase is dependent on insulin, and glycogen synthesis occurs at a rate that is 10-30% lower than in the first rapid phase (Jentjens et al. 2001).

9.4.3 Effects of protein hydrolysates and free amino acids on post-exercise glycogen synthesis

van Loon et al. (2000b) investigated whether an increase in carbohydrate intake, ingestion of a mixture of protein hydrolysate and amino acids in combination with carbohydrate, or both results in higher postexercise muscle glycogen synthesis rates than does ingestion of $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ carbohydrate, provided at 30-min intervals.

Eight trained cyclists visited the laboratory 3 times, during which a control beverage and 2 other beverages were tested. After the subjects participated in a strict glycogen-depletion protocol, muscle biopsy samples were collected. The subjects received a beverage every 30 min to ensure ingestion of 0.8 g carbohydrate $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Carb trial), 0.8 g carbohydrate $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ plus 0.4 g wheat protein hydrolysate plus free leucine and and phenylalanine $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Carb + Pro trial), or 1.2 g carbohydrate $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Carb + Carb trial). After 5 h, a second biopsy was taken.

Plasma insulin response in the Carb + Pro and Carb + Carb trial were higher than those in the Carb trial. Muscle glycogen synthesis was higher in both trials than in the Carb trial (35.4 ± 5.1 and 44.8 ± 6.8 compared with 16.6 ± 7.8 μmol glycosol units · g dry wt^{-1} · h^{-1}, respectively; $P < 0.05$) (**Figure 25**).
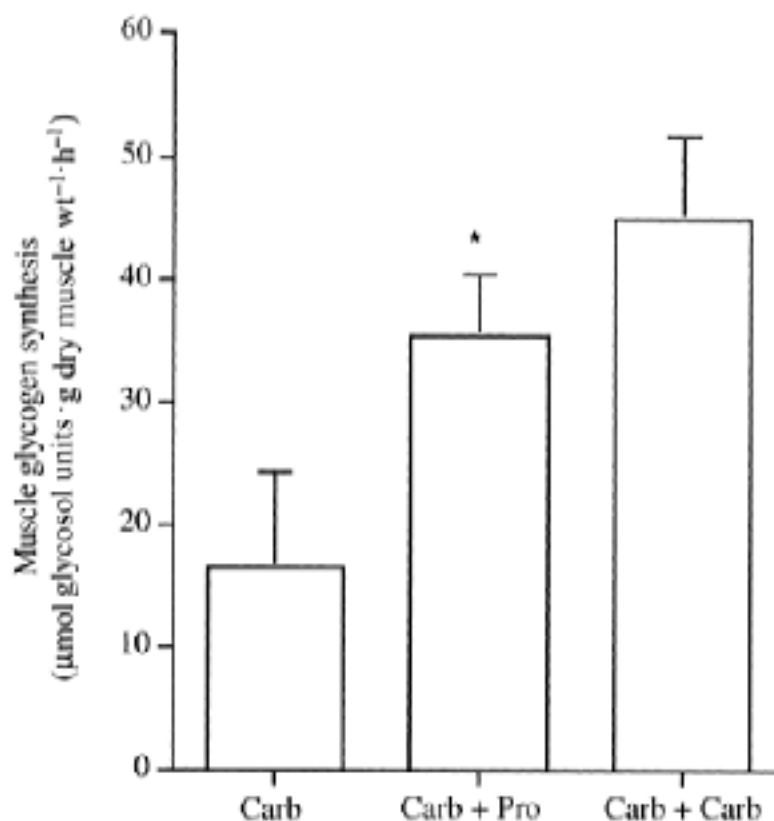
Figure 25. Mean (±SEM) Muscle Glycogen Synthesis Rate After Ingestion of the Control Drink (carbohydrate only; Carb) and Drinks Containing Carbohydrate Plus Wheat-Protein Hydrolysate Plus Free Leucine and Phenylalanine (Carb + Pro) or an Isoenergetic Amount of Carbohydrate (Carb + Carb). *Significantly higher than Carb, $P < 0.05$. *Data from* van Loon et al. 2000b.

Thus, addition of a mixture of protein hydrolysate and amino acids to carbohydrate-containing solution can stimulate glycogen resynthesis. However, glycogen synthesis can also be accelerated by increasing carbohydrate intake when supplements are provided at 30-min intervals.

The aim of recent study by Jentjens et al. (2001) was to investigate whether coingestion of protein also increases muscle glycogen synthesis when 1.2 g CHO · kg⁻1 · h⁻¹ is ingested. Eight male cyclists performed two experimental trials separated by 1 wk. After glycogen-depleting exercise, subjects received either CHO (1.2 g · kg⁻¹ · h⁻¹) or CHO + Pro (1.2 g CHO · kg⁻¹ · h⁻¹ + 0.4 g Pro · kg⁻¹ · h⁻¹) during a 3-h recovery period.

Muscle biopsies were obtained immediately, 1 h, and 3 h after exercise. Blood samples were collected immediately after the exercise bout and every 30 min thereafter. Plasma insulin was significantly higher in the CHO + Pro trial compared with the CHO trial ($P < 0.05$). No difference was found in plasma glucose or in rate of muscle glycogen synthesis between the CHO and the CHO + Pro trials.

The results of this study suggest that a further increase in the insulin concentration by additional supplementation of protein and amino acids does not increase the rate of glycogen synthesis when CHO intake is sufficient and supplemented at regular intervals of ≤ 30 min. Insulin can, therefore, be excluded as the limiting factor for glycogen synthesis.

The total amount of carbohydrate intake post-exercise, on the other hand, seems to play a more important role when maximal rates of muscle glycogen synthesis are required. An intake of 1.2 g · kg⁻¹ · h⁻¹ or more seems to be required to achieve the maximal glycogen resynthesis rate (Jentjens et al. 2001).

10 Amino acid mixtures

Since the 1989 ban on tryptophan for supplements in the United States, the number of amino acid mixtures as dietary supplements has dropped (Bucci and Unlu 2000). The comparative higher cost and poorer organoleptic properties of free-form amino acid mixtures relative to high-quality grades of protein and protein hydrolysates have also contributed to their decline in popularity.

Moreover, there is no evidence that shows any advantage to using the much more expensive free-form amino acids simply to augment protein intake. However, there are times when the use of amino acid mixtures may be more useful than either high-protein foods or intact protein supplements.

10.1 Effects of amino acid mixtures on muscle protein synthesis

10.1.1 Postexercise supplementation

Tipton et al. (1999) examined the response of net muscle protein synthesis to ingestion of amino acids after a bout of resistance exercise. In this well-controlled study, a primed, constant infusion of L-[ring-^{2}H$_5$]phenylalanine was used to measure net muscle protein balance in three male and three female volunteers on three occasions.

Subjects consumed in random order 1 liter of *1)* a mixed amino acids (40 g) solution (MAA), *2)* an essential amino acid (40 g) solution (EAA), and *3)* a placebo solution (PLA). Arterial amino acid concentrations increased approximately 150-640% above baseline during ingestion of MAA and EAA. Net muscle protein balance was significantly increased from negative during PLA ingestion (-50 ± 23 nmol · min^{-1} · 100 ml leg volume^{-1}) to positive during MAA ingestion (17 ± 13 nmol · min^{-1} · 100 ml leg volume^{-1}) and EAA (29 ± 14 nmol · min^{-1} · 100 ml leg volume^{-1}; *P* < 0.05).
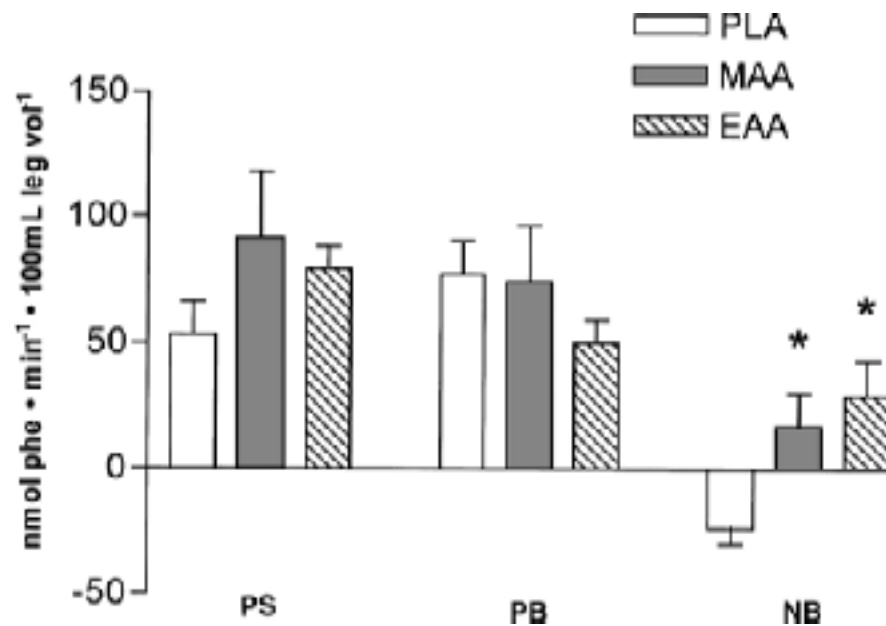
Figure 26. Muscle Protein Synthesis (PS), Protein Breakdown (PB), and Net Muscle Protein Balance (NB) After Resistance Exercise During Consumption of Solution of Placebo (PLA), 40 g of Mixed Amino Acids (MAA), and 40 g of Essential Amino Acids (EAA). *Significantly different from PLA. *Data from* Tipton et al. 1999.

Because net balance was similar for MAA and EAA, it does not appear necessary to include nonessential amino acids in a formulation designed to elicit an anabolic response from muscle after exercise.

Authors concluded that ingestion of oral essential amino acids results in a change from net muscle protein degradation to net muscle protein synthesis after heavy resistance exercise in humans similar to that seen when the amino acids were infused. Thus, *these results suggest that hyperaminoacidemia from ingestion of oral amino acids is an effective method of maximizing the anabolic effect of exercise.*

It has not been clear that oral administration of amino acids after exercise would have the same effect as infusion on protein metabolism. First-pass splanchnic uptake of amino acids account for a large portion (from 20 to 90%, depending on amino acid) of ingested amino acids (for references see Tipton et al. 1999).

Furthermore, splanchnic protein breakdown is increased during exercise (Williams et al. 1996). Because the splanchnic bed would have the first access to ingested amino acids after exercise, the amount of amino acids from an oral solution available to the peripheral tissues, i.e., skeletal muscle, after exercise may be reduced.

Nevertheless, authors concluded that "***oral amino acid supplement is just as effective as amino acid infusion for producing hyperaminoacidemia and net muscle protein synthesis***."

From the present data it is not possible to determine the potential mehanism(s) that is responsible for the increased anabolic response after exercise with amino acid ingestion. Possible candidates include hormones, paracrine substances, and vasodilators. Insulin has been demonstrated to increase muscle protein anabolism. However, in the present study there was no significant difference in insulin levels among any of the three treatments.

Intracellular amino acid availability has been demonstrated to have an anabolic effect on muscle in two studies (Bennett et al. 1989; Svanberg et al. 1996). Furthermore, Biole et al. (1997) showed that hyperaminoacidemia, similar to that found in the present study, and exercise have an additive effect on muscle protein balance.

In the study by Tipton et al. (1999), muscle intracellular amino acids concentrations were increased by amino acid ingestion. Thus, it is likely that increased availability of amino acids for protein synthesis was the primary mechanism for the increase in muscle protein anabolism observed when subjects consumed amino acids. Alternatively, one or more individual essential amino acids may initiate muscle protein synthesis.

However, I am unaware of any research indicating that amino acid preparations taken orally are, over the course of a day, more effective than amino acids consumed as natural components of protein-rich foods.

Nevertheless, companies that market amino acid supplements for athletes indicate that amino acids found in food are liberated slowly in the digestive processes, somewhat like a time-release tablet, and may not elicit similar effects compared to consumption of free-form amino acids.

10.1.2 Studies reporting no anabolic effect from post-exercise amino acid supplementation

The purpose of study by Williams et al. (2001) was to examine the effects on strength gains of ingesting glucose-amino acid supplementation immediately following resistance exercise.

Seven untrained participants with a median age of 23 years and mean (SD) body mass 68.9 (13.5) kg resistance trained on a leg extension machine for five days a week for 10 weeks, using four sets of 10 repetitions. Alternate legs were trained on successive days, one leg each day. Subjects ingested either a supplement including 0.8 g glucose $\cdot$ kg^{-1} and 0.2 g amino acids $\cdot$ kg^{-1}, or placebo, on alternate training days immediately after training. Therefore the supplement was always ingested after training the same leg (supplement leg).

Isometric, isokinetic, and 1 repetition maximum (RM) strength were measured before, during, and after training. Blood samples were analyzed to determine the acute responses of insulin and glucose to resistance exercise and supplementation or placebo.

Serum insulin concentration peaked 20 minutes after supplement ingestion at nine-fold the placebo level, and remained significantly elevated for at least 80 minutes ($P < 0.01$). Isometric, isokinetic, and 1 RM strength improved on both supplement and placebo legs ($P < 0.05$). There were no significant differences in the gain in strength between the supplement leg and the placebo leg ($P > 0.05$).

Authors concluded that regular glucose-amino acid supplementation immediately after resistance exercise is unlikely to enhance the gain in muscle strength brought about by resistance training. However, as Kreider (2002b) pointed out, there are several limitations with this study that make it difficult to reach the conclusions drawn by the authors:

• As the authors point out at the end of their paper, the experimental design assumed that the nutritional intervention employed would only affect the supplemented leg and have no influence on the placebo leg. This assumption may not have merit for several reasons.

First, it is well known that when you exercise the same muscle group on one side of the body, approximately 25 % of the nerve impulses are also sent to the other side of the body. This cross-lateral innervation means that even though the subjects trained one leg each day, both legs were actually being trained to some degree.

Second, this design assumes that the potential increase in protein synthesis in response to glucose-amino acid supplementation would only influence the exercised leg. However, there is no evidence that the improved anabolic response to exercise would only affect the exercise leg and have no influence on the non-exercised leg. Therefore, it is possible the glucose-amino acid supplementation benefited both legs to some degree.

• This study only evaluated seven subjects. It is possible that the small sample size may have masked a true effect from the supplementation protocol. Additionally, both men and women were used as subjects and there were more men in the training group and more women in the control group. Using a mixed cohort of subjects increases the variability in results because of differences in strength, size, and hormonal responses to resistance exercise.

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- There is no data to show that performing four sets of 10 repetitions of leg extension on one leg two or three times per week will produce a catabolic or an anabolic hormonal response. Additionally, as authors point out, there is no data showing that the volume of training employed in this study would be sufficient to promote muscle hypertrophy.

    Thus, it would have been more appropriate to use a more traditional training stimulus (i.e., a periodized, multiple-exercise, multiple-set program performed three to four times per week). Since this was not done, extrapolating results to athletes engaged in a traditional multiple exercise resistance-training program is inappropriate.

- Study by Tipton et al. (1999) used *essential* amino acids with carbohydrates. The present study administered approximately 55 grams of carbohydrates with 14 grams of an amino acid mixture; 17% glutamine, 11%, leucine, 10.3% aspartic acid, and less than 10% of 15 other amino acids.

10.1.3 Pre-exercise supplementation

Another excellent study by Tipton et al. (2001) was designed to determine whether consumption of an oral essential amino acid-carbohydrate supplement (EAC) before exercise results in a greater anabolic response than supplementation after resistance exercise.

Six healthy human subjects participated in two trials in random order, PRE (EAC consumed immediately before exercise), and POST (EAC consumed immediately after exercise). A primed, continuous infusion of L-[ring-^{2}H$_5$] phenylalanine, femoral arteriovenous catheterization, and muscle biopsies from the vastus lateralis were used to determine phenylalanine concentrations, enrichments, and net uptake across the leg.

Study Protocol
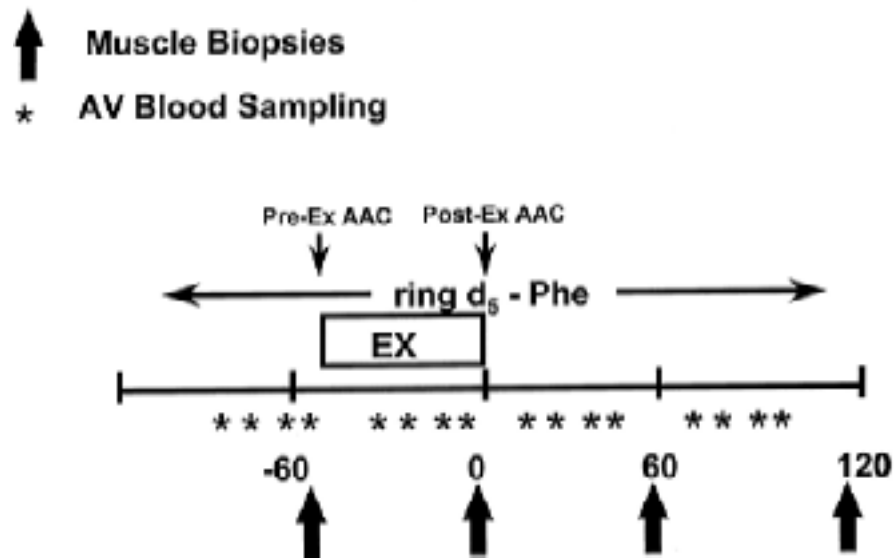
Figure 27. Schematic Representation of the Study Protocol. Time values are in minutes from the end of exercise. AV = arteriovenous; EX = exercise; EAC = essential amino acid-carbohydrate (supplement). *Data from* Tipton et al. 2001.

Blood and muscle phenylalanine concentrations increased by approximately 130% after drink consumption in both trials. Amino acid delivery to the leg was increased during exercise and remained elevated for the 2 h after exercise in both trials. Delivery of amino acids (amino acid concentration times blood flow) was significantly greater in PRE than in POST during the exercise bout and in the 1st h after exercise ($P < 0.05$). Total net phenylalanine uptake across the leg was greater ($P = 0.0002$) during PRE (209 ± 42 mg) than during POST (81 ± 19). Phenylalanine disappearance rate, an indicator of muscle protein synthesis from blood amino acids, increased after EAC consumption in both trials.
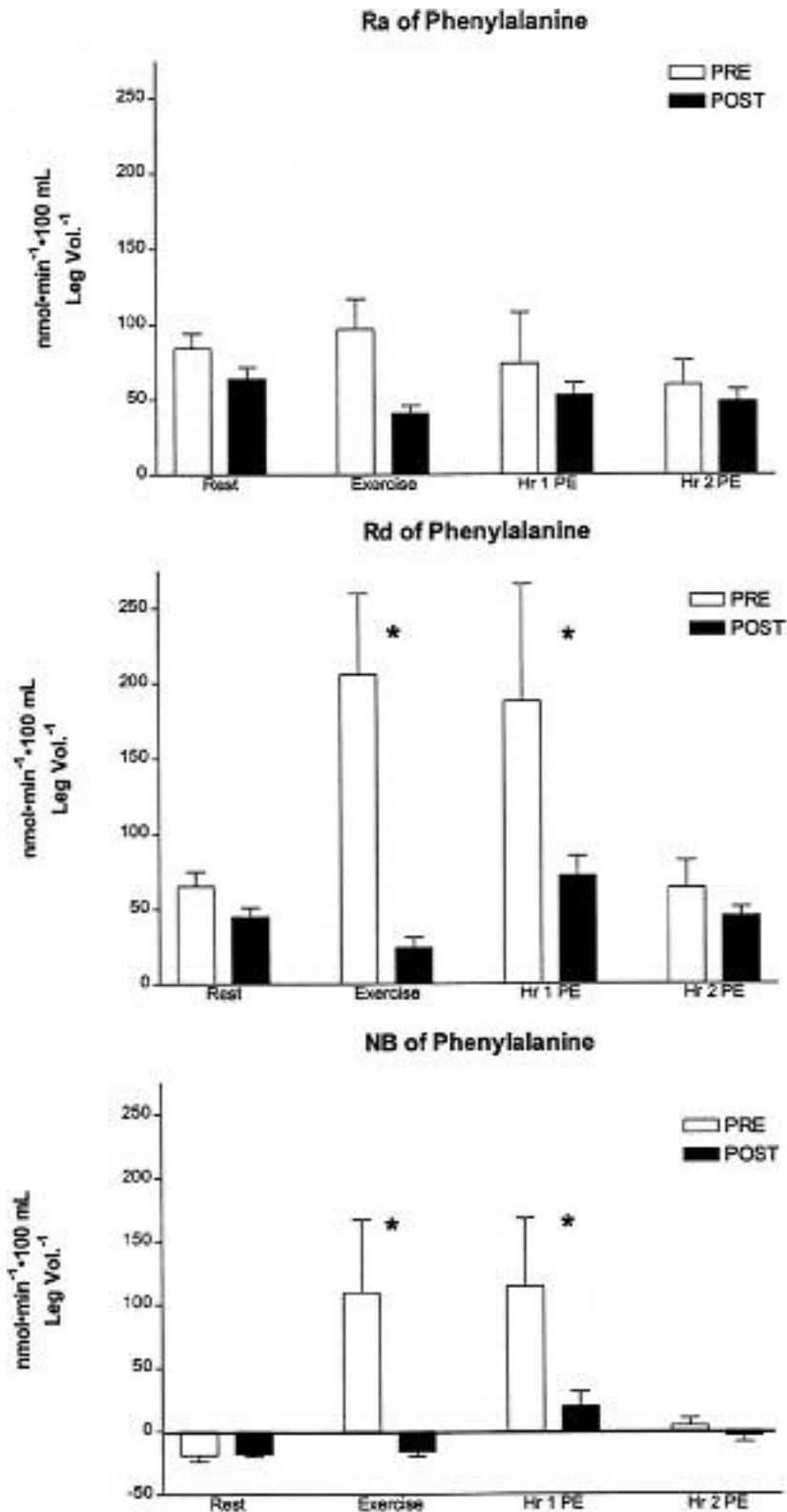
Figure 28. Muscle Phenylalanine Rate of Appearance in Muscle (R_a), Phenylalanine Uptake from Blood (R_d), and Net Phenylalanine Balance Across Leg (NB) from 4 Time Periods during PRE (open bars) and POST (solid bars). Rest = mean of 3 resting values; Ex = mean of 4 samples taken during resistance exercise; Hr 1 PE = mean of 4 samples taken during the 1st h after exercise. Hr 2 PE = mean of 3 samples taken during the 2nd h after exercise. *PRE significantly different from POST, $P < 0.05$. *Data from* Tipton et al. 2001.

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These results indicate that the response of net muscle protein synthesis to consumption of an EAC solution immediately before resistance exercise is greater than that when the solution is consumed after exercise.

According to authors, it is likely that the greater delivery to the muscle during PRE accounts for the greater net uptake than during POST. During exercise in the POST trial, net muscle protein balance, as well as phenylalanine R_d, and index of muscle protein synthesis, was unchanged, whereas in the PRE trial, phenylalanine R_d and NB were increased. Consuming a source of amino acids before exercise increases amino acid availability. Providing amino acids at a time when blood flow is elevated, such as during exercise bout, maximizes delivery to the muscle.

10.2 Effects of amino acid mixture on body composition and exercise performance

Antonio et al. (2000) determined the effects of 6 wk of EAA (10 g provided isoleucine, leucine, valine, lysine, methionine, phenylalanine, thereonine, tryptophan at 1.483, 1.964, 1.657, 1.429, 0.699, 1.289, 1.111, 0.368, respectively) supplementation on body composition and exercise performance in untrained women ($n = 21$). Subjects were randomly assigned to a placebo (cellulose) or an EAA (average daily dose of 18.3 g of EAA in pill form) group.

Each subject participated in aerobic and heavy-resistance training three times per week. Body composition was assessed via dual x-ray absoprtiometry analysis. Muscular endurance was determined via treadmill time to exhaustion, and strength was assessed by the total amount of weight lifted from one set to exhaustion at an estimated 12 repetitions maximum.

No changes occurred in either group for body weight for body weight, lean body mass, fat mass, or bone mineral content. Treadmill time to exhaustion (TTE) improved significantly ($P < 0.05$) in the EAA group (mean ± SD; pre-

TTE = 13.15 ± 3.67 min, post-TTE = 14.73 ± 4.26 min), whereas the placebo group did not change significantly. The total weight lifted at the subject´s maximum 12 repetitions did not significantly change in either group.

Thus, in previously untrained individuals, the ingestion of EAA combined with aerobic and heavy-resistance training for 6 wk did not have a significant effect on body composition or muscular strength; however, aerobic muscular performance increased significantly.

In fact, the EAA group´s improvement in treadmill time to exhaustion was more than twice that of the placebo group. According to the authors, performance enhancement seen in the EAA group could be due to the provision of an added fuel source during exercise.

10.3 Brief review of selected individual amino acids

10.3.1 Glutamine

Candow et al. (2001) recently reported that glutamine supplementation (0.9 g · kg lean mass tissues^{-1} · day^{-1}) during resistance training has no significant effect on muscle performance, body composition or muscle protein degradation in young healthy adults. However, there appeared to be a trend towards a greater myofibrillar breakdown in the placebo group (**Figure 29**), but the difference was not significant.
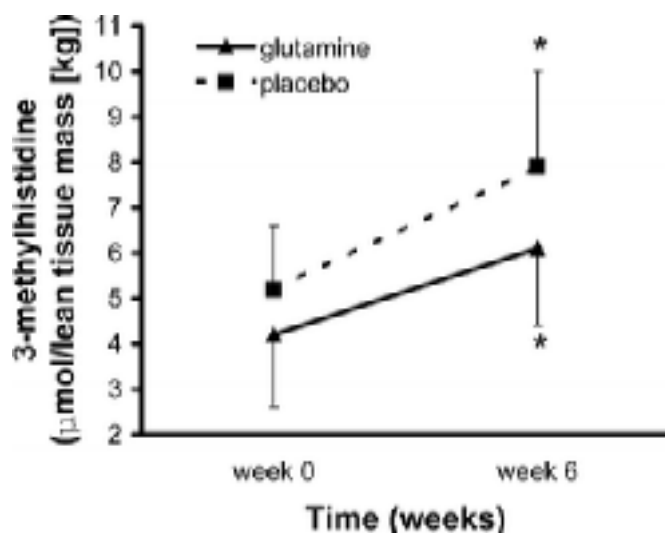
Figure 29. **Mean Urinary 3-Methylhistidine Levels for Glutamine and Placebo Groups at Week 0 and Week 6 of Resistance Training**. 3-methylhistidine, which occurs only in the myofibrillar proteins, is often used as indicator of contractile protein degradation. Error bars are ± SEM. *$P < 0.05$ compared to week 0. *Data from* Candow et al. 2001.

The dose of glutamine used in this study was rather large, approximately 45 g · day^{-1}. It could be argued that excessive doses of any compound can have inhibitory effect, but this cannot be determined from the current study. None of the subjects reported negative side effect while taking glutamine supplement. Authors concluded that strength training may not be stressful enough to benefit from glutamine supplementation.

10.3.2 Arginine and ornithine

Two studies that reported greater increases in body mass and reduction in percentage of body fat (Elam et al. 1988) and gains in total body strength and lean body mass (Elam et al. 1989) as a result of arginine and ornithine supplementation were seriously flawed by inappropriate statistical analysis. In both studies, it appears that multiple dependent variables were included in a single analysis variance and used as levels of an independent variable (Chromiak and Antonio 2002).

10.3.3 Branched chain amino acids (BCAA)

Blomstrand et al. (1991a, 1991b) have focused on the administration of BCAA as a means of delaying central fatigue during prolonged activities (**Figure 30**), such as marathon racing, cross-county ski racing, and soccer matches. When 7.5-21 g of BCAA were administered before and during exercise, small improvements were reported in both physical (1991a) and mental performance (1991b) in some subjects.

Rest
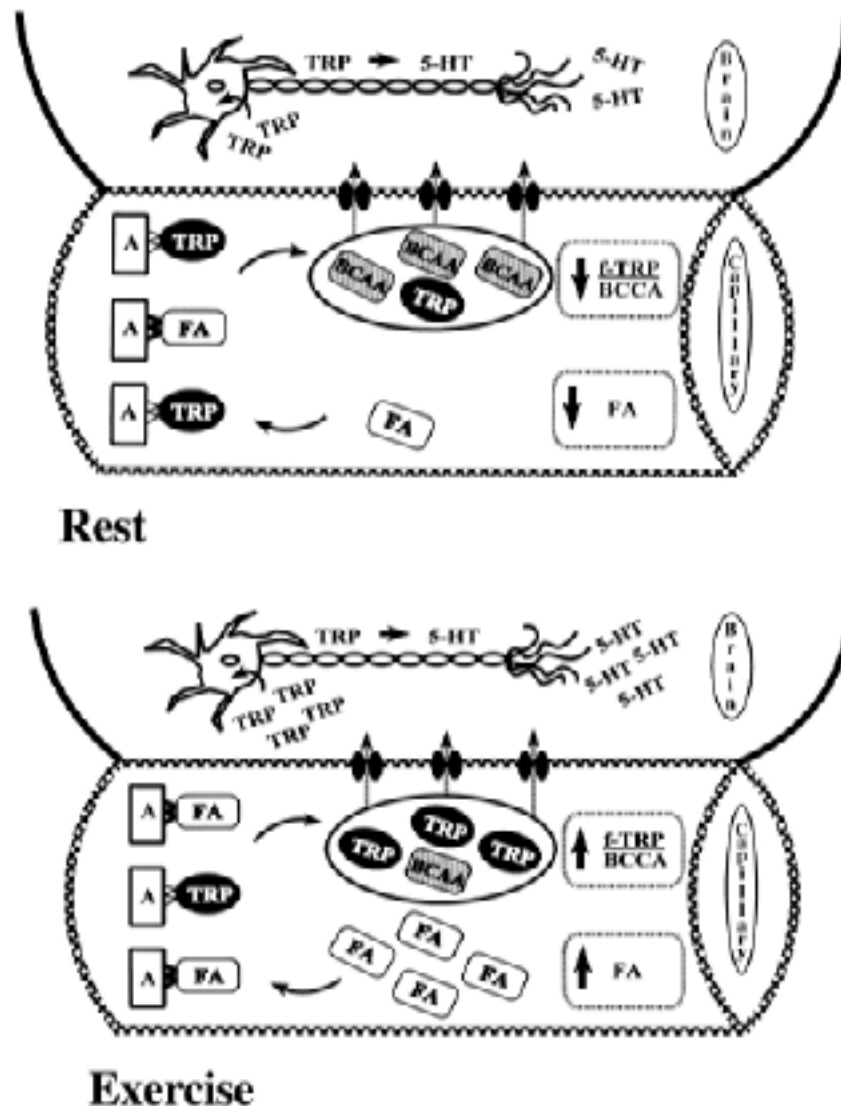
Exercise

Figure 30. **Proposed Effects of Carbohydrate (CHO) and Branched Chain Amino Acids (BCAA) on Central Fatigue During Prolonged Exercise**. Serotonin (5-hydroxytryptamine, 5-HT) is formed in the body by hydroxylation and decarboxylation of the essential amino acid tryptophan (TRP). Increased synthesis of 5-HT occurs in response to an increase in delivery of blood-borne TRP. Most of the TRP in blood circulates loosely bound to albumin; however, unbound, or free, TRP (f-TRP) is transported across the blood-brain barrier. *This transport occurs via specific receptors that TRP shares with other large neutral amino acids, most notably BCAA. Thus, 5-HT synthesis in brain increases when there is an increase in the ratio of the fTRP concentration in blood plasma to the total BCAA concentration in plasma (i.e., when f-TRP:BCAA rises).* This increase was proposed to occur during prolonged exercise for two reasons*: 1)* BCAA are taken up from blood and oxidized for energy during contraction of skeletal muscle, and *2)* fatty acid (FA) concentrations in plasma increase, causing a parallel increase in plasma f-TRP because fatty acids displace TRP from its binding sites on albumin. *Increased synthesis of 5-HT has been reported to induce sleep, depress motor neuron excitability, and alter autonomic and endocrine function* (Kreider 1998)*. Consequently, exercise-induced increases in 5-HT have been suggested to affect tiredness, psychological perception of fatigue, muscle power output, and hormonal regulation during exercise*. It has also been hypothesized that chronic elevations in 5-HT concentrations, that may occur in athletes maintaining high volume training, may explain many of the reported signs and symptoms of the overtraining syndrome. *Data from* Davis et al. 2000.

It should be noted, however, that although field studies such as these are designed to mimic the real-world situations of athletes, such studies are often limited in scientific value. For example,

- Subjects are often not appropriately matched to prevent inherent differences in the performance capacities of the group being assigned to control and experimental groups.

- Studies of this nature often do not, or cannot, blind subjects to experimental treatments to prevent bias on the part of the subjects toward the treatment that they believe to be better.

- These studies often fail to control important variables, i.e., exercise intensity and food and water intake, across the treatment groups.

In well-controlled laboratory experiments, the administration of BCAA showed to have no benefits on performance during prolonged bouts of exercise (Blomstrand et al. 1995; van Hall et al. 1995).

According to Kreider and Leuholtz (2001), the greatest potential application of BCAA supplementation is to help athletes tolerate training to a greater degree rather than a performance enhancement supplement. For detailed review see Kreider 1998.

11 Fortified protein supplements

Ingredients most commonly used to fortify protein supplements are carbohydrates, amino acids, creatine, vitamins, minerals, and herbs/plant extracts.

11.1 Effects of protein-carbohydrate supplementation on glycogen synthesis

In a well-known study by Zawadzki et al. (1992), carbohydrate, protein, and carbohydrate-protein supplements were compared to determine their effects on muscle glycogen storage during recovery from prolonged exhaustive exercise.

Nine male subjects cycled for 2 h on three separate occasions to deplete their muscle glycogen stores. Immediately and 2 h after each exercise bout, they ingested 112.0 g carbohydrate (CHO), 40.7 g protein (Pro), or 112.0 g carbohydrate and 40.7 g protein (CHO + Pro). Blood samples were drawn before exercise, immediately after exercise, and throughout recovery. Muscle biopsies were taken from the vastus lateralis immediately and 4 h after exercise.

During recovery the plasma glucose response of the CHO treatment was significantly greater than that of the CHO + Pro treatment, but the plasma insulin response of the CHO + Pro treatment was significantly greater than that of the CHO treatment. Both the CHO and CHO + Pro treatments produced plasma glucose and insulin responses that were greater than those produced by the Pro treatment.

The rate of muscle glycogen storage during the CHO + Pro treatment [35.5 ± 3.3 (SE) mumol $\cdot$ g protein^{-1} h^{-1}] was significantly faster than during the CHO treatment (25.6 ± 2.3 mumol $\cdot$ g protein^{-1} $\cdot$ h^{-1}), which was significantly faster than during the Pro treatment (7.6 ± 1.4 mumol $\cdot$ g protein^{-1} $\cdot$ h^{-1}).

According to authors, the results suggest that post-exercise muscle glycogen storage can be enhanced with a carbohydrate-protein supplement as a result of the interaction of carbohydrate and protein on insulin secretion. However, non-isoenergetic supplements made the interpretation of these findings difficult. Nevertheless, they suggested that Pro and CHO supplements were more efficacious in promoting glycogen resynthesis than was CHO alone.

In a recent study by Niles et al. (2001), ten endurance-trained males were studied to investigate the ergogenic effects of isocaloric carbohydrate (CHO, 152.7 g) and carbohydrate-protein (CHO + Pro, 112 g CHO with 40.7 g Pro) drinks ingested after a glycogen lowering diet and exercise bouts. Treatments were administered in a double-blind and counterbalanced fashion.

After a glycogen-depleting run, two dosages of a drink were administered with a 60 min interval between dosages. The CHO + Pro trial resulted in higher serum insulin levels (60.84 vs. 30.1 $\mu U \cdot ml^{-1}$) 90 min into recovery than the CHO only trial ($P < 0.05$).

Furthermore, the time to run to exhaustion was longer during the CHO + Pro trial (540.7 ± 91.56 sec) than the CHO only trial (446.1 ± 97.09 sec, $P < 0.05$). The intensity used for the performance run was set at a VO_2 level of 10 ± 3.0% above the individual anaerobic threshold. Within this intensity range, intramuscular glycogen is though to be the primary fuel for muscular activity (Brooks and Mercier 1994).

Therefore, since blood glucose was similar between trials and the run time to exhaustion was longer following the CHO + Pro trial, the assumption may be made that the greater hyperinsulinemia of the CHO + Pro trial stimulated a more rapid post-exercise muscle glycogen synthesis and a larger muscle glycogen store prior to the performance run.

However, the intramuscular measures of glycogen were not directly assessed. Nevertheless, authors concluded that recovery process of muscle glycogen seems to be accelerated when a drink which contains adequate amounts of both CHO and Pro is consumed compared to an isocaloric drink which only contains CHO. According to authors, this nutritional strategy may be critical for athletes who need to engage in multiple events or training sessions during the course of day.

11.2 Effects of protein-carbohydrate supplementation on hormonal responses (testosterone, GH, IGF-1)

Kraemer et al. (1998) studied hormonal responses to consecutive days of heavy-resistance exercise with or without carbohydrate-protein supplementation (Mass Fuel, Twin Laboratories, Ronkonkoma, NY).

The supplement was composed of 33% protein (hydrolysed casein and albumin) and 67% carbohydrate (glucose polymers, glucose, crystalline fructose, and xylitol). One serving of supplement also contained between 50 and 1,000% of USA RDA for all essential vitamins and minerals. The placebo was specifically designed to look and taste identical to the supplement while providing minimal carbohydrate, protein, and calories.

In this study, nine resistance-trained men consumed either a protein-carbohydrate supplement or placebo for 1 wk in a crossover design separated by 7 days. The last 3 days of each treatment, subjects performed resistance exercise. The supplement was consumed 2 h before and immediately after the workout, and blood was obtained before and after exercise (0, 15, 30, 45, and 60 min post-exercise).

Lactate, growth hormone, and testosterone were significantly elevated immediately post-exercise. The lactate response was significantly lower during supplementation on *days 2* and *3*. Growth hormone and prolactin response on *day 1* were significantly higher during supplementation. After exercise, testosterone declined below resting values during supplementation. Cortisol

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decreased immediately post-exercise on *day 1*; the response was diminished on *days 2* and *3*. Glucose and insulin were significantly elevated by 30 min during supplementation and remained stable during placebo. Insulin-like growth factor-I was higher during supplementation on *days 2* and *3*.

In summary, these data indicate that consuming a nutritional supplement before and immediately after heavy-resistance training workouts performed over 3 consecutive days results in different exercise-induced patterns of metabolic and hormonal variables.

Specifically, consuming a protein-carbohydrate supplement before and after a resistance training sessions increases the concentrations of glucose, insulin, growth hormone, and IGF-I while decreasing lactate accumulation. Such responses would be predicted to enhance glycogen and protein synthesis during recovery; however, this was not determined in this investigation.

11.3 Effects of nutritional supplements designed to promote lean tissue accretion

Kreider et al. (1996) examined the effects of ingesting nutritional supplements designed to promote lean tissue accretion on body composition alterations during resistance training.

Twenty-eight resistance-trained males blindly supplemented (28 days) their diets with maltodextrin, high-calorie protein carbohydrate supplement containing chromium picolinate and boron (Gainers Fuel 1000, Twinlaboratories, Inc., Ronkonkoma, NY), or vitamin-mineral fortified carbohydrate-protein supplement containing 20 g · day^{-1} creatine monohydrate (Phosphagain, Experimental & Applied Sciences, Inc., Golden, CO).

No significant differences were observed in absolute or relative total body water among groups. Energy intake and body weight significantly increased in all groups combined throughout the study with no group or interaction differences observed. Dual energy x-ray absorptiometry-determined body mass

significantly increased in each group throughout the study with significantly greater gains observed in the Gainers Fuel 1000 and Phosphagain groups. Lean tissue mass (excluding bone) gain was significantly greater in the maltodextrine group, while fat mass and percent body fat were significantly increased in the Gainers Fuel 1000 group.

Results indicate that total body weight significantly increased in each group and that Phosphagain supplementation resulted in significantly greater gains in lean tissue mass during resistance training.

In another well-controlled study by Kreider et al. (1999), 51 college football players were matched and randomly assigned to supplement their diet with either a carbohydrate placebo, a vitamin-mineral fortified carbohydrate-protein supplement (Met-Rx, Met-Rx Substrate Technologies, Inc., Newport Beach, Ca), a vitamin-mineral fortified carbohydrate-protein supplement containing $20 \text{ g} \cdot \text{day}^{-1}$ creatine monohydrate (Phosphagain, Experimental & Applied Sciences, Inc., Golden, CO), or a vitamin-mineral fortified carbohydrate-protein supplement containing $25 \text{ g} \cdot \text{day}^{-1}$ creatine monohydrate (Phosphagain 2, Experimental & Applied Sciences, Inc., Golden, CO) during 84 days of winter resistance/agility training. Additionally, a group of 10 subjects maintained normal dietary practices during training and served as non-supplemented controls.

Total body weight, total body water, DEXA determined body composition, and isotonic strength tests were assessed on days 0, 35, and 84 of training. The results revealed that mean gains in soft tissues/lean mass were significantly greater in the Phosphagain and Phosphagain 2 groups than changes in the non-supplemented, carbohydrate, and Met-Rx groups (non-supplemented 0.7 ± 1.3; carbohydrate 1.2 ± 1.6; Met-Rx 0.8 ± 1.2; Phosphagain 2.3 ± 1.4; Phosphagain 2 3.4 ± 1.8 kg)

In addition, mean gains in 1RM bench press in Met-Rx, Phosphagain and Phosphagain 2 groups were significantly greater than gain observed in the non-supplemented group (non-supplemented 2.0 ± 9; placebo 7.6 ± 7; Met-Rx 9.8 ± 6; Phosphagain 10.3 ± 5; Phosphagain 2 10.0 ± 8 kg).

While it is unclear which individual or combination of nutrients was responsible for the results observed, the theoretically active nutrients included creatine monohydrate, glutamine, and taurine.

11.3.1 Brief review of creatine supplementation

Creatine (methylguanidine-acetic acid), a nitrogenous amine, is a naturally occuring constituent in food. In humans, total creatine stores approximate 120 g in the average-sized adult male (70 kg), with corresponding smaller and larger amounts in individuals who weight less or more.

The daily turnover rate of creatine to creatinine has been estimated to be about 1.6% of the total creatine pool (Balsom et al. 1995). Based on measurements of renal excretion of creatinine, the daily requirements for creatine supplied through the diet or from endogenous synthesis in a 70 kg man approximate $2 \text{ g} \cdot \text{day}^{-1}$ (Williams et al. 1999).

It should be noted, however, that since many athletes are larger than 70 kg and since intense training promotes protein degradation, serving to increase serum and urinary creatinine levels, larger athletes undergoing intense training may have a greater daily creatine turnover and requirement (e.g., $2 \text{ to } 3 \text{ g} \cdot \text{day}^{-1}$) (Williams et al. 1999).

Creatine is an osmotically active substrate; thus an increase in intracellular total creatine concentration as free creatine and phosphocreatine may induce the influx of water into the cell, increasing intracellular water and, concomitantly, body mass (Volek et al. 1997a, 1997b).

Moreover, some research suggests that increased cellular hydration and/or increased phosphocreatine may stimulate protein synthesis or decrease protein degradation, possibly increasing fat free mass (Volek and Kraemer 1996; Clark 1997; Volek et al. 1997a, 1997b).

12 The fast and slow protein concept

12.1 Theoretical basis

Different types of protein have different time courses of amino acid release. For example, since casein clumps when exposed to acid in the stomach, it is digested at a relatively slow rate, which results in a modest but prolonged increase in amino acids in the blood (Beaufrere et al. 2000). On the other hand, whey protein is comprised of a mixture of soluble proteins that are digested rather rapidly, resulting in a more pronounced but shorter increase in amino acids in the blood (Beaufrere et al. 2000).

The time course of amino acid release following ingestion of a protein has been shown to affect protein metabolism and synthesis. For example, Boirie et al. (1997) compared the effects of ingesting 30 g of whey (fast protein) and 43 g of casein (slow protein) on protein utilization and synthesis. The amount of whey and casein evaluated was chosen to match the proteins for leucine content, since the researchers used labeled leucine methodology to assess protein use and synthesis rates.

The researchers found that whey protein ingestion promoted a greater increase in amino acid levels in the blood and a greater rate of protein storage during the first two hours after feeding compared to casein. However, the rate of protein storage returned to baseline within three to four hours after feeding. In addition, whey protein had no effect on the rate of protein breakdown.

Casein ingestion promoted a more modest but prolonged increase in amino acid concentrations that were greater than the whey group during three to six hours after feeding. This resulted in a greater amount of protein storage, as well as less protein breakdown.

The studies described above have served as the theoretical basis for various nutritional supplement companies to develop "time releasing" and "slow protein" formulations. As pointed out by Kreider (2002a), however, there are several points to consider when interpreting results of these studies:

• These studies only evaluated the effects on protein synthesis of consuming different types of protein. As the researchers point out, it is unclear how co-ingesting fast or slow proteins with other nutrients (e.g., carbohydrate, fat, etc.) may affect protein synthesis rates (Beaufrere et al. 2000).

• The primary differences between the fast and slow proteins on protein synthesis were observed between three and seven hours after ingesting the slow protein meal. Athletes engaged in intense training typically ingest four to six meals per day, as well as snack between meals in order to provide adequate caloric intake.

• Although there is some theoretical basis for consuming slow proteins, there is no evidence that ingesting casein promotes greater gains in strength and muscle mass during training than whey protein.

However, it is possible that ingestion of slow proteins may help athletes maintain muscle mass to a better degree when dieting or when observing prolonged fasting periods.

12.2 Studies comparing effects of fast and slow proteins

Demling and Desanti (2000) compared the effects of a moderate hypocaloric, high-protein diet and resistance training, using two different protein supplements, versus hypocaloric diet alone on body compositional changes in overweight police officers.

A randomized, prospective 12-week study was performed comparing the changes in body composition produced by three different treatment modalities in three study groups. One group ($n = 10$) was placed on a nonlipogenic, hypocaloric diet alone (80% of predicted needs). A second group ($n = 14$) was placed on the hypocaloric diet plus resistance exercise plus a high-protein intake (1.5 g · kg body mass^{-1} · day^{-1}) using a casein supplement (Met-Rx, Met-Rx USA, Irving, CA). In the third group ($n = 14$) treatment was identical to the second, except for the use of a whey protein (Pro Score, Champion Nutrition, Concord, CA).

Investigators found that weight loss was approximately 2.5 kg in all three groups. Mean percent body fat with diet alone decreased from a baseline of 27 ± 1.8 to 25 ± 1.3% at 12 weeks. With diet, exercise and casein the decrease was from 26 ± 1.7 to 18 ± 1.1% and with diet, exercise and whey protein the decrease was from 27 +/- 1.6 to 23 +/- 1.3%. The mean fat loss was 2. 5 ± 0.6, 7.0 ± 2.1 and 4.2 ± 0.9 kg in the three groups, respectively.

Lean mass gains in the three groups did not change for diet alone, versus gains of 4 ± 1.4 and 2 ± 0.7 kg in the casein and whey groups, respectively. Mean increase in strength for chest, shoulder and legs was 59 ± 9% for casein and 29 ± 9% for whey protein, a significant group difference.

According to authors, this significant difference in body composition and strength is likely due to improved nitrogen retention and overall anticatabolic effects caused by the peptide components of the casein.

However, there are several limitations with this study that make it difficult to reach the conclusions drawn by the authors. For example, although Pro Score is essentially whey protein supplement, the Met-Rx supplement contains a blend of proteins (milk protein isolates, caseinate, whey protein, egg white) and a host of other nutrients.

To my knowledge, Met-Rx contains relatively high amounts of added glutamine, and high-dose glutamine supplementation may maintain positive nitrogen balance in hypocaloric diet (Rosena et al. 1999). Thus, it is impossible to attribute results observed solely to ingesting of casein.

13 Protein intake and bone health

13.1 Basic facts about bone physiology

The body of young adult human contains approximately 1100 g of calcium. 99% of the calcium is in the skeleton. Throughout life, bone is being constantly resorbed and new bone is formed. The calcium in bone turns over at a rate of 100% per year in infants and 18% per year in adults (Ganong 2001c).

Bone remodeling is mainly a local process carried out in small areas by populations of cells called bone-remodeling units. First, osteoclasts resorb bone, and then osteoblast lay down new bone in same general area. Adequate amounts of protein, minerals, and vitamins must be available for the maintenance of normal bone structure.

13.2 Hormonal control of calcium metabolism

Three hormones are primarily concerned with the regulation of calcium metabolism:

- *Parathyroid hormone* (PTH). PTH acts directly on bone to increase bone resorption and mobilize Ca^{2+}. In addition to increasing the plasma Ca^{2+} and depressing the plasma phosphate, PTH increases phosphate excretion in the urine. PTH also increases reabsorption of Ca^{2+} in the distal tubules, although Ca^{2+} excretion is often increased in hyperparathyroidism because the increase in the amount filtered overwhelms the effect of reabsorption.

  Circulating ionized calcium acts directly on the parathyroid glands in a negative feedback fashion to regulate the secretion of PTH. When the plasma Ca^{2+} level is high, PTH secretion is inhibited and the Ca^{2+} is deposited in the bone. When it is low, secretion is increased and Ca^{2+} is mobilized from the bones.

- *Calcitonin*. Calcitonin lowers the circulating calcium and phosphate levels. It exerts its calcium-lowering effect by inhibiting bone resorption. This action is direct, and calcitonin inhibits the activity of osteoclasts *in vitro*. It also increases Ca^{2+} excretion in the urine.

  Measurement of circulating calcitonin by immunoassay indicates that it is not secreted until the plasma calcium level reaches approximately 9.5 mg · dL^{-1} and that above this calcium level plasma calcitonin is directly proportionate to plasma calcium (Ganong 2001c).

- *Calcitriol* (1,25-dihydroxycholecalciferol). In addition to increasing Ca^{2+} absorption from the intestine, calcitriol facilitates Ca^{2+} reabsorption in the kidneys. It acts on bone, where it mobilizes Ca^{2+} and PO_4^{3-}, by increasing the number of mature ostoeclasts. It also stimulates osteoblasts, but the net effect is still Ca^{2+} mobilization.

  The formation of calcitriol in the kidneys is regulated in a feedback fashion by plasma Ca^{2+} and PO_4^{3-}. Its formation is facilitated by PTH, and when the plasma Ca^{2+} level is low, PTH secretion is increased. When the plasma Ca^{2+} level is high, little calcitriol is produced. This effect of Ca^{2+} on production of calcitriol is the mechanism that brings about adaptation of Ca^{2+} absorption from the intestine.

13.3 Effects of high-protein and low-protein diet on bone health

Increasing dietary protein increase urine calcium excretion such that for each 50 g increment of protein consumed, and extra 60 mg of urinary calcium is excreted (Kerstetter and Allen 1994). It follows that the higher the protein intake, the more urine calcium is lost and the more negative calcium balance becomes. Since 99% of the body´s calcium is found in bone, one would hypothesize that high protein induced hypercalciuria would results in high bone resorption and increased prevalence of osteopenia or osteoporotic-related fractures.

The epidemiological and clinical data addressing this hypothesis are controversial. On one hand, most (Geinoz et al. 1993; Michaelsson et al. 1995; Cooper et al. 1996), but not all (Metz et al. 1993) epidemiological studies found a positive association between protein intake and bone mineral density (BMD). On the other hand, many (Abelow et al. 1992; Feskanich et al. 1996; Meyer et al. 1997), but not all (Munger et al. 1999) report higher fractures in groups consuming a high protein diet.

Clinical intervention trials generally support the hypothesis. Most, but not all report an increase in bone resorption when animals or humans were fed a high protein diet. A summary of the controversy that surrounds the influence of dietary protein on calcium metabolism and bone health was presented by Massey and colleagues (Barzel and Massey 1998; Heaney 1998; Massey 1998).

There is growing evidence that a low protein diet has a detrimental effect on bone. Kerstetter et al. (1997) reported that in healthy young women, acute intakes of a low-protein diet (0.7 g protein $\cdot$ kg^{-1}) decreased urinary calcium excretion with accompanied secondary hyperparathyroidism. The etiology of the secondary hyperparathyroidism is due, in part, to a significant reduction in intestinal calcium absorption during a low protein diet (Kerstetter et al. 1998).

In a recent short-term intervention trial, Kerstetter et al. (2000) evaluated the effects of graded levels of dietary protein (0.7, 0.8, 0.9, and 1.0 g protein $\cdot$ kg^{-1}) on calcium homeostasis. Secondary hyperparathyroidism developed by day 4 of the 0.7 and 0.8 g protein $\cdot$ kg^{-1} diets (due to the decreased intestinal calcium absorption), but not during the 0.9 or 1.0 g protein $\cdot$ kg^{-1} diets in eight young women (**Figure 31**).
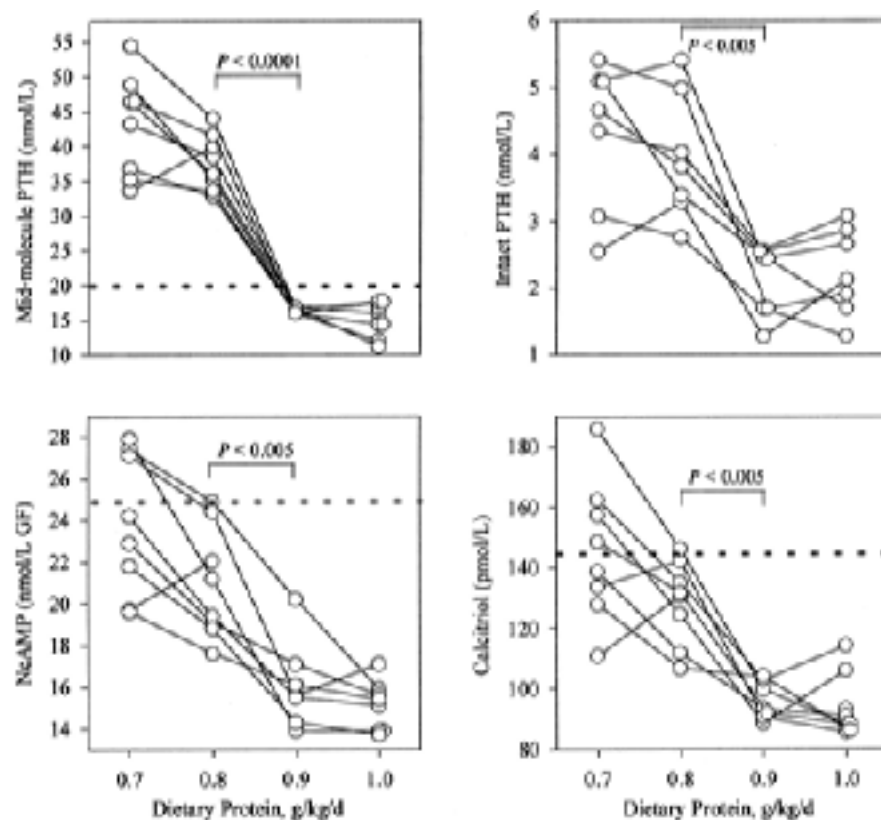
Figure 31. Individual Responses in Calcitropic Hormones at Day 4 in Response to Graded Intakes of Dietary Protein ($n = 8$ healthy young women). The order of treatments was randomized. The upper limits of normal are represented by horizontal dashed lines. Human PTH is a linear polypeptide with a molecular weight of 9500 that contains 84 amino acids. PTH is synthesized as part of a larger molecule containing 115 amino acid residues (preproPTH). Upon entry of preproPTH into the endoplasmic reticulum, a leader sequence is removed from the amino terminal to form 90-amino-acid polypeptide proPTH. Six additional amino acid residues are removed from the amino terminal of proPTH in the Golgi apparatus, and the 84-amino-acid polypeptide PTH is packaged in secretory granules and released as the main secretory product of the chief cells. The normal plasma level of intact PTH is 10-55 pg · mL^{-1} (Ganong 2001c). The half-life of PTH is approximately 10 minutes, and the secreted polypolypeptide is rapidly cleaved by the Kupffer cells in the liver into midregion and carboxyl terminal fragments that are probably biologically inactive. Since many of the old radioimmunoassays used antibodies against the midregion of the molecule, they measured the fragments as well as the intact hormone and hence gave values that were falsely high. To get around this problem, two-site immunoassays have been developed, using one antibody against the amino terminal and another against the carboxyl terminal. *In secondary hyperparathyroidism, high level of PTH occurs as a compensation for hypocalcemia rather than as a primary abnormality of the parathyroid gland*. Vitamin D$_3$ (cholecalciferol) is produced in the skin of mammals from 7-dehydrocholesterol by the action of sunlight. In the liver, vitamin D$_3$ is converted to 25-hydroxycholecalciferol. The 25-hydroxycholecalciferol is converted in the cells of the proximal tubulus of the kidneys to the more active metabolite 1,25-dihydroxycholecalciferol (calcitriol). The normal level of calcitriol is approximately 100 pmol · L^{-1} (Ganong 2001c) Vitamin D$_3$ and its derivatives are secosteroids; i.e., they are steroids in which one of the rings has been opened. In this case, it is the B ring. *Calcitriol is a hormone because it is produced in the body and transported in the bloodstream to produce effects in target cells*. NcAMP = nephrogenous cyclic adenosine monophosphate, a bioindicator of PTH activity. GF = glomerular filtration. *Data from* Kerstetter et al. 2000.

There were no significant differences in mean urinary calcium excretion over the relatively narrow range of dietary protein intakes studied, although the mean value with the 0.7-g $\cdot$ kg^{-1} intake was lower than that with the 1.0-g $\cdot$ kg^{-1} intake by 0.25 mmol (10 mg). According to authors, the lack of change may be due to the small sample and the inherent variability in urinary calcium excretion.

Similarly, when Giannini et al. (1999) restricted dietary protein to 0.8 g protein $\cdot$ kg^{-1}, he observed an acute rise in serum PTH in 18 middle-aged hypercalciuric adults. Taken together, both of studies suggest, at least in the short term, that RDA for protein (0.8 $\cdot$ kg^{-1}) does not support normal calcium homeostasis.

The long-term consequences of restricted protein intake on calcium and bone metabolism are unknown but could potentially be an important and unrecognized problem. Analysis of available data from the US Department of Agriculture indicated that 31% of women aged $\geq$ 20 y consume less protein than the 1989 RDA (Food Surveys Research Group 1998). Only half of these women (i.e., 15% of women aged $\geq$ 20 y) considered their own diets to be too low in dietary protein (US Department of Agriculture 1997).

Overall, it appears that both low and high protein diet may be detrimental to bone health. Low protein diet interferes with intestinal calcium absorption and IGF-1 levels, and high protein diets induce excess urine calcium loss.

13.4 Exercise and bone health

One of the best strategies for preventing post-menopausal osteoporosis is to maximize bone mass during childhood and premenopausal adulthood. This can be accomplished by practicing regular exercise and insuring adequate lifetime calcium intake. Habitual physical activity, particularly high-impact activities such as running and gymnastics, are associated with higher lifetime peak bone density and lower fracture risk.

Other factors to consider while developing osteoporosis-prevention strategies include the following (American College of Sports Medicine 1995):

- *Bone adaptations are exercise-specific.* Lower body exercise will strengthen leg bones but will not strengthen bones in the upper body.

- *Bone growth occurs when they are overloaded.* Nonweight bearing exercise, such as swimming, has no effect on bone mass.

- *Gains in bone mass are reversible.* Changes in bone mass are lost with subsequent deconditioning.

- *People with lowest bone mass will improve the most from an exercise program.*

- *People gave a genetic ceiling that limits their capacity to increase bone mass.* Gains in bone mass plateau as this ceiling is approached.

Limited evidence suggest that physical activity increases PTH release in young, middle-aged, and older individuals, an effect that may contribute to the positive effects of exercise on bone mass.

For example, one study exercised six subjects on bicycle ergometers at different intensities for 10 minutes (Brahm et al. 1997). Blood samples were analyzed for ionized calcium, total calcium, calcitonin, pH, and plasma PTH levels. Moderate exercise (50% VO_{2max}) initially depressed PTH levels, whereas near-maximal effort elevated hormone concentration during and in recovery from exercise.

PTH release and subsequent calcium mobilization may provide the osteogenic raw materials that allow the mechanical forces from the exercise to produce effects on skeletal mass and density.

14 High-protein intake and kidneys

Because of the kidney´s role in processing and ridding the body of nitrogenous waste, this organ could be particularly susceptible to damage from being overworked. Theoretically, large amounts of nitrogen from a high-protein diet may become toxic.

Despite its role in nitrogen excretion, *there are presently no data in the peer-reviewed scientific literature demonstrating the normal kidney will be damaged by the increased demands of protein consumed in quantities above the RDA.*

Furthermore, real world examples support this contention since kidney problems are nonexistent in the bodybuilding community in which high-protein intake has been the norm for over half a century (Street 2001).

14.1 Very-high-protein diet and healthy athletes

The study by Poortmans and Dellalieux (2000) investigated body-builders and other well-trained athletes with high- and medium-protein intake, respectively, in order to shed light on this issue.

The athletes underwent a 7-day nutrition record analysis as well as blood sample and urine collection to determine the potential renal consequences of a high protein intake. The data revealed that despite higher plasma concentration of uric acid and calcium, bodybuilders had renal clearances of creatinine, urea, and albumin that were within the normal range.

The nitrogen balance for both groups became positive when daily protein intake exceeded $1.26 \text{ g} \cdot \text{kg}^{-1}$ but there were no correlations between protein intake and creatinine clearance, albumin excretion rate, and calcium excretion rate.

To conclude, *it appears that protein intake under 2.8 g · kg^{-1} does not impair renal function in well-trained athletes as indicated by the measures of renal function used in this study.*

15 Protein intake and diabetic nephropathy

15.1 Basic facts about diabetic nephropathy

Diabetic nephropathy is caused by a combination of an abnormal regulation of the vasomotor tone of the afferent-efferent arterioles and the effect of hyperglycemia and other toxins in the glomerulus. The afferent arterioles are vasodilated by means of prostaglandins, bradykinins, and other mediators while the efferent arterioles are vasoconstricted by thromboxane A_2 resulting in a state of hyperfiltration which, coupled with high levels of glucose and other byproducts produced under conditions of insulin resistance, causes thickening of the basal membrane, resulting in glomerulosclerosis (Leutholtz and Ripoll 1999).

The earliest detectable abnormality is microalbuminuria. Normal urinary albumin excretion is < 20 µg $\cdot$ min^{-1}: values between 20 and 200 µg $\cdot$ min^{-1} constitute microalbuminuria (Marshall 2000). This progresses through clinical proteinuria (> 200 µg min^{-1}) with gradually declining filration rate and increasing plasma creatinine concentration to frank uraemia (Marshall 2000). Ideally, all diabetic patients (except young children and the very old) should have their albumin excretion measured annually.

15.2 Protein intake and development of diabetic nephropathy

In seven studies, dietary protein intake was reported to be similar in patients with diabetes with and without nephropathy (for references see Franz et al. 2002). In all studies, protein intake was in the range of usual intake and rarely exceeded 20% of the energy intake.

In an cross-sectional study of 2500 type 1 diabetic subjects, those who reported protein consumption $< 20\%$ of total energy had average albumin excretion rates < 20 µg $\cdot$ min^{-1} (Toeller et al. 1997). Those in whom protein intake was $\geq 20\%$ of daily energy (22% of patients) had average albumin excretion rates > 20 µg $\cdot$ min^{-1}. In individuals with macroalbuminuria, 32%

consumed > 20% of total energy from protein versus 23% for those with microalbuminuria and 20% for those with normal albumin excretion. This suggests that a high-protein intake may have detrimental effect on renal function.

According to American Diabetes Association (2002), there is no evidence to suggest that usual protein intake (15-20% of total calories) should be modified if renal function is normal. The long-term effects of consuming > 20% of energy as protein on the development of nephropathy has not been determined, and therefore it may be prudent to avoid protein intakes > 20% of total daily energy (American Diabetes Association 2002).

16 High-protein intake and serum lipid profile

16.1 Basic facts about lipoproteins

16.1.1 "Bad" cholesterol

Among the lipoproteins, the low-density lipoproteins (LDL) that normally carry between 60 to 80% of the total serum cholesterol have the greatest affinity for the cells of the arterial wall. LDL delivers cholesterol to arterial tissue where the LDL particles become *1)* oxidized to alter their physiochemical properties and *2)* taken up by macrophages inside the arterial wall to initiate atherosclerosis plaque development.

16.1.2 "Good" cholesterol

Unlike LDL, high-density lipoproteins (HDL) exert a protective effect against heart disease. HDL acts as a scavenger in the reverse transport of cholesterol by removing it from the arterial wall and delivering it to the liver for incorporation into bile and subsequent excretion via the intestinal tract.

16.2 High-protein diet and lipid profile

There have been some concerns about the adverse effects of high-protein diets on the serum lipid profile. However, it would seem that these concerns have little basis in facts.

In one study a diet higher in lean animal protein, including beef, was found to result in more favorable HDL and LDL levels (Wolfe and Giovannetti 1991). The study involved 10 moderately hypercholesterolemic subjects (6 women, 4 men). They were randomly allocated to isocaloric high- or low-protein diets for four to five weeks, after which they switched over to the other.

Protein provided either 23% or 11% of energy intake; carbohydrate provided 65% or 53%; and fats accounted for 24%. During high-protein diet, mean fasting plasma total cholesterol, LDL, and triglycerides were significantly lower, HDL was raised by 12%, and the ratio of LDL to HDL consistently decreased.

17 Other possible problems of high-protein diet

Price et al. (1994) have shown that high protein diets, once initiated, should be maintained to preserve lean mass. Essentially, what has been shown is that high protein diets, while they may result in high fed gains (i.e., stimulation of protein synthesis), there is a concomitant high fasting loss of protein. These findings have implications for athletes who habitually consume high dietary protein intakes; they run the "risk" of losing lean body mass, if they do not consistently follow this practice (Phillips 2002).

Furthermore, consumption of dietary protein in excess of the physiological needs can result in compromising the carbohydrate status of athletes and can adversely affect their performance during training and competition.

Also, at least 20% energy should be provided by fat in the diets of athletes (Jonnalagadda 2002), given the role of fat in providing energy for athletes involved in prolonged, low-intensity activity. Restricting fat intake to less than 15% of energy intake is not advisable, because it will not only limit performance by inhibiting intramuscular triglyceride storage, which is a significant source of energy during activities of all intensities, but will also affect important physiological functions (Jonnalagadda 2002).

18 Very-high-protein diets and weight reduction

The popularity of diet books promoting high-protein intakes with emphasis on some form of carbohydrate restriction is of concern to informed health professionals because of lack of scientific evidence to support their claims and their long-term adverse implication for overall health (Freedman 2000).

A meal of pure protein elicits a thermic effect nearly 25% of the meals total caloric value. The relatively large calorigenic effect of ingested protein has been used by some to advocate a high-protein diet for weight reduction. It is maintained that because of protein´s relatively high thermic effect, fewer calories ultimately become available to the body compared with a meal of similar caloric value consisting mainly of lipid and carbohydrates.

Although this point has some merit, one must consider other factors when formulating a sound weight-loss program, particularly for physically active individuals. Important considerations include a very-high-protein diet´s potential for *1)* strain on liver and kidney function and accompanying dehydration, *2)* electrolyte imbalance, *3)* glycogen depletion, and *4)* lean tissue loss.

A high-carbohydrate diet that includes fruits, vegetables, nonfat dairy products, and whole grains has been shown to lower blood pressure (Appel 1997), so limitation of these foods may raise blood pressure via associated reduction in potassium, calcium, and magnesium coupled with increased sodium intake.

Furthermore, high-protein foods such as meat, poultry, seafood, eggs, seeds, and nuts are high in purines. Purines are broken down into uric acid, so excess consumption of these foods increases uric acid levels and may cause gout in susceptible individuals (Franzese 2000).

A recent review of literature regarding the effects of low-carbohydrate (high-protein) diets reported from 1956 to 2000 concluded from 20 studies that there is a pattern of weight loss that ranges from 2.8 to 12.0 kg within varying time frames and number and type of subjects included (Freedman 2000).

However, when carbohydrate is restricted, subjects generally reduce their overall intake of calories, and this calorie deficit is related to the weight loss. These studies raised important questions regarding the long-term effects of these diets on weight maintenance and overall health.

19 Summary

- A variety of studies using both humans and animals have shown that the rate of protein synthesis is generally depressed during exercise. After exercise, protein synthesis increases for periods up to 48 hours before declining to baseline values.

  This has been shown following both endurance and resistance exercise, although the increase in protein synthesis with resistance exercise tends to be greater than what is observed following endurance exercise.

- Human muscle can oxidize at least seven amino acids: leucine, isoleucine, valine, glutamate, asparagine, aspartate and alanine. Of these amino acids, however, oxidation of only the branched chain amino acids (leucine, isoleucine and valine, BCAA) appears to be increased during catabolic states such as exercise.

  The increased BCAA oxidation during exercise is due to the synergistic effects of a high abundance of BCAA in skeletal muscle protein along with the fact that the activity of the enzymes responsible for the transamination and subsequent oxidation are also relatively high in muscle.

- Generally, athletes feel felt that their protein needs are substantially greater than the recommendations from nutrition scientists (RDA) – both opinions could be correct as the latter is based on data from essentially sedentary subjects.

- Recent scientific studies suggest a variety of factors need to be considered when determining protein requirements, including but probably not limited to total energy, carbohydrate availability, exercise intensity, exercise duration, exercise type, dietary protein quality, training history, gender, age, and timing of nutrient intake.

- These studies indicate that for physically active individuals protein intake needs could be as high as 1.6-1.8 g · kg^{-1} (approximately twice the current recommendation).

    However, the testimonials of athletes who believe that their success depends on consumption of large amounts of protein and energy, and the examples of Japanese sumo wrestlers and Olympic weight lifters, suggest that further laboratory investigations are necessary before the question of protein need in those attempting to increase lean mass is settled.

- Despite these increased protein needs, assuming energy intake is sufficient to match the additional expenditures of training and competition (which can be excessive), special protein supplementation is unnecessary for most who consume a varied diet containing complete protein foods (meat, fish, eggs and dairy products).

- Those at greatest risk of consuming insufficient protein are those whose lifestyle combines other factors known to increase protein needs with a regular exercise program, e.g., those with insufficient energy intake (dieters), growing individuals, vegetarians, the elderly, those with muscle diseases and so on.

- No evidence indicates that commercial protein powders promote muscle growth any more effectively than protein consumed in a well-balanced diet. Thus, consumers are assailed with hypothetical advantages or disadvantages of different proteins extrapolated from animal studies or enteral/parenteral clinical nutrition field.

- Several studies have shown that protein hydrolysates made up mostly of di- and tripeptides are absorbed more rapidly than amino acids and protein hydrolysates containing larger peptides, and much more rapidly than whole foods.

    The considerable greater absorption rate of amino acids from the dipeptide than from the amino acid mixture appears to be the result of

uptake by a system that has a greater transport capacity than amino acid carrier system, thus minimizing competition among its substrates.

- There is evidence that oral intake of protein hydrolysates and amino acids in combination with carbohydrates can result in an insulinotropic effect as much as 100% greater than with the intake of carbohydrates only.

    However, the results of the recent study suggest that a further increase in the insulin concentration by additional supplementation of protein and amino acids does not increase the rate of glycogen synthesis, when carbohydrate intake is sufficient and supplemented at regular intervals of $\leq$ 30 min. Insulin can, therefore, be excluded as the limiting factor for glycogen synthesis.

- There is some evidence that oral ingestion of essential amino acids results in a change from net muscle protein degradation to net muscle protein synthesis after heavy resistance exercise in humans similar to that seen when the amino acids were infused. Thus, these results suggest that hyperaminoacidemia from ingestion of oral amino acids is an effective method of maximizing the anabolic effect of exercise.

    Furthermore, there is some evidence that the response of net muscle protein synthesis to consumption of an oral essential amino-acid carbohydrate solution immediately before exercise is greater than when the solution is consumed after exercise.

- Recent data suggests that glutamine supplementation during resistance training has no significant effect on muscle performance, body composition or muscle protein degradation in young healthy adults.

- There is no evidence based on properly conducted, rigorous scientific studies that oral supplementation of specific amino acids induces growth hormone release that, in conjunction with resistance training, increases muscle mass and strength to a greater extent than resistance exercise alone.

- Recent data have confirmed that soy protein is complete for humans, and has similar biological value, as illustrated by PDCAAS score, in humans to milk, beef, and egg proteins.

  Furthermore, soy protein isolates contain isoflavone glucosides; mostly genistin and diadzin. Isoflavones have been studied in postmenopausal osteoporosis and generally found to have positive effect in maintaining bone density and reducing fractures.

  The most consistently demonstrated beneficial effect of soy has been on lipids. A meta-analysis of 38 published controlled human clinical trials, of an average of 47 g daily soy protein consumption (primarily in subjects with hyperlipidemia), noted significant reduction in total and LDL cholesterol and triglycerides, with the hypocholesterolemic effect significantly related to pretreatment cholesterol levels.

- Increasing dietary protein increase urine calcium excretion such that for each 50 g increment of protein consumed, and extra 60 mg of urinary calcium is excreted. It follows that the higher the protein intake, the more urine calcium is lost and the more negative calcium balance becomes.

  Since 99% of the body´s calcium is found in bone, one would hypothesize that high protein induced hypercalciuria would results in high bone resorption and increased prevalence of osteopenia or osteoporotic-related fractures. However, the epidemiological and clinical data addressing this hypothesis are controversial.

- There is growing evidence that a low protein diet has a detrimental effect on bone. Recent studies suggest, at least in the short term, that the RDA for protein ($0.8 \cdot kg^{-1}$) does not support normal calcium homeostasis.

- For persons with diabetes, there is no evidence to suggest that usual protein intake (15-20% of total calories) should be modified, if renal function is normal.

Acknowledgment

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