7 Muscle Mass and Weight Gain Nutritional Supplements

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Abstract
There are numerous sports supplements available that claim to increase lean body mass. However, for these sports supplements to exert any favorable changes in lean body mass, they must influence those factors regulating skeletal muscle hypertrophy (i.e., satellite cell activity, gene transcription, protein translation). If a given sports supplement does favorably influence one of these regulatory factors, the result is a positive net protein balance (in which protein synthesis exceeds protein breakdown). Sports supplement categories aimed at eliciting a positive net protein balance include anabolic hormone enhancers, nutrient timing pre- and postexercise workout supplements, anticatabolic supplements, and nitric oxide boosters. Of all the sports supplements available, only a few have been subject to multiple clinical trials with repeated favorable outcomes relative to increasing lean body mass. This chapter focuses on these supplements and others that have a sound theoretical rationale in relation to increasing lean body mass.

Key words
Sports nutrition · Lean body mass · Creatine · Protein supplements · HMB · Nitric oxide · Anabolic · Anticatabolic · Nutrient timing

1. INTRODUCTION

To appreciate fully how certain nutritional supplements increase lean body mass, a thorough understanding of the structural, systemic, and molecular processes that are responsible for such increases in lean body mass is needed. Although there is still a lot to be determined and understood relative to how skeletal muscle is increased, research scientists for the most part have

From: *Nutritional Supplements in Sports and Exercise*
Edited by: M. Greenwood, D. Kalman, J. Antonio,
DOI: 10.1007/978-1-59745-231-1_7, © Humana Press Inc., Totowa, NJ

agreed on several key components that are absolutely necessary for such adaptations to occur. Some of these components are satellite cell activity, muscle-specific gene transcription, protein translation, and nutrient (amino acid) transport into the skeletal muscle. In addition, growth factors/anabolic hormones including testosterone, growth hormone, insulin-like growth factor-1 (IGF-1), and insulin are also necessary for increases in skeletal muscle mass.

Although some sports supplements have been repeatedly observed to increase skeletal muscle mass, they are at best utilized as a complement to an optimally periodized resistance training program. In fact, the greatest stimulus for muscle hypertrophy is mechanical stress in the form of resistance training. The main question to ask relative to sports supplements is whether a given supplement is able to augment the stimulus of resistance training so muscle hypertrophy is maximized. An overview of how skeletal muscle hypertrophy is regulated follows.

2. IMPORTANCE OF NET PROTEIN BALANCE

The functional component of skeletal muscle is comprised of two primary proteins: actin and myosin. Of the two, myosin is the primary protein that increases in size. Hence, when exercise biochemists observe changes in muscle mass from a cellular frame of reference, myosin is often the protein of interest. From a general perspective, muscle hypertrophy can be summarized by the status of *net protein balance*. Net protein balance is equal to muscle protein synthesis minus muscle protein breakdown. For skeletal muscle hypertrophy to occur, the net protein balance must be positive (synthesis must exceed breakdown). At rest, in the absence of an exercise stimulus and nutrient intake, the net protein balance is negative *(1–4)*. As previously stated, resistance training is essential for creating the stimulus necessary for skeletal muscle hypertrophy to occur. However, when resistance training is performed alone, in the absence of nutritional and supplemental interventions, net protein balance still does not increase to the point of becoming anabolic. Specific nutrients and supplements noted later in the chapter are needed in conjunction with the resistance training for the net protein balance to become positive.

3. ROLE OF GENES IN SKELETAL MUSCLE HYPERTROPHY

As already mentioned, net protein balance has two components: synthesis and breakdown. To understand how sports supplements can increase protein synthesis, an understanding of the biochemical process is needed. The center of all bodily functions (including the addition of skeletal muscle) is at the level of genes. Specific to hypertrophy, it is the genes that must be expressed as the proteins in skeletal muscle (i.e., myosin and actin). For instance, once muscle-specific genes are activated, they are copied into messenger RNA (mRNA), which is specific to certain proteins in cells. Once mRNA is transcribed, it is then translated into actual proteins. Using myosin as an example, what must first happen is an increase in activation of the myosin gene. Once the myosin gene is activated, is it copied into myosin mRNA. It is this myosin mRNA that then directs the process of changing amino acids into polypeptides and ultimately a functional myosin protein that is added to the existing matrix of the sarcomere. The point at which the myosin protein is synthesized (from the addition of amino acids under the direction of mRNA), the myosin gene is said to be "expressed." The reason why all of this tedious biochemical information is necessary is that any supplement that claims to increase muscle mass must in some way influence one of these aforementioned variables. For instance, some supplements increase growth hormone, which has been associated with increases in IGF-1. IGF-1, in turn, increases the activity of certain cell-signaling pathways, which may increase muscle-specific (i.e., myosin) gene transcription. Other sports supplements may increase lean body mass by increasing the rate at which amino acids are synthesized into muscle proteins under the direction of mRNA. Yet other sports supplements may increase the delivery of nutrients (i.e., amino acids, glucose) to contracting skeletal muscle, which conceivably results in greater substrate from which lean body mass is acquired. Each of these mechanisms and the supplements that may enhance these contributions to skeletal muscle hypertrophy are discussed. Following is a discussion of one of the best and traditional sports supplements—protein.

4. PROTEIN SUPPLEMENTS

When attempting to increase lean body mass, an essential component equal to a sound resistance training program is protein consumption. Not only is protein intake required for skeletal muscle hypertrophy, protein is also needed to repair damaged cells and tissue and for a variety of metabolic and hormonal activities. Protein is the only macronutrient that contains nitrogen. Given the importance of attaining a positive nitrogen balance, it is vitally important that protein be ingested on a daily (and meal-to-meal) basis. When discussing protein as a nutritional supplement, two main questions arise: 1) How much protein is required for an individual engaging in resistance training? 2) What are the types of protein supplements and which are the best sources of protein?

4.1. Protein Requirements

One of the most controversial subjects in the science of sports nutrition has been protein intake. The main controversy and divided opinions have focused on the safety and effectiveness of protein intake currently recommended by the recommended daily allowance (RDA). Currently, the RDA for protein in healthy adults is 0.8 g/kg body weight per day *(5)*. This recommendation accounts for individual differences in protein metabolism, variations in the biological value of protein, and nitrogen losses in the urine and feces. When determining the amount of protein that needs to be ingested to increase lean body mass, many factors must be considered, such as protein quality, energy intake, carbohydrate intake, the amount and intensity of the resistance training program, and the timing of the protein intake. Although 0.8 g/kg/day may be sufficient to meet the needs of nearly all non-resistance-trained individuals, it is likely not sufficient to provide substrate for lean tissue accretion or for the repair of exercise-induced muscle damage *(6,7)*. In fact, many clinical investigations indicate that individuals who engage in physical activity/exercise require higher levels of protein intake than 0.8 g/kg/day regardless of the mode of exercise (i.e., endurance, resistance) *(8–12)* or training state (i.e., recreational, moderately or well trained) *(13–15)*. So the question that remains: How much protein *is* required for individuals engaging in resistance training and wanting to increase lean body mass? General recommendations for individuals who engage in strength/power exercise range from 1.6 to 2.0 g/kg/day *(6,13–16)*. A protein intake at these levels help ensure that the net protein balance remains positive, a prerequisite for skeletal muscle hypertrophy to occur.

4.2. Types of Protein Supplement

Although protein can be obtained from whole foods, many resistance trained athletes supplement their diet with protein containing supplements (e.g., protein powders, meal replacements drinks, sports bars). Advances in food processing technology have allowed for the isolation of high quality proteins from both animal and plant sources. Other reasons for supplementing the diet with protein supplements include convenience, simplicity, and the fact that protein supplements also have other benefits, such as a longer shelf life than whole food sources in addition to being more cost-effective in many cases.

Ingesting protein at 1.6 to 2.0 g/kg/day is not the only parameter to consider, however, because it is also important to note that not all protein is the same. Different types of protein are composed of varying amounts of amino acids, which serve as the building blocks of protein. There are approximately 20 amino acids that can be used to make proteins (Table 1). There are eight essential amino acids that must be obtained from the diet because the body cannot synthesize these amino acids. There are also approximately six conditionally essential amino acids that the body has difficulty synthesizing, and therefore individuals are primarily dependent on dietary sources for these amino acids. The body can easily synthesize the remaining amino acids, so they are considered nonessential. Not all protein sources contain the same amounts of amino acids. Protein is classified as *complete* or *incomplete* depending on whether it contains adequate amounts of the essential amino acids. Animal sources of protein contain all essential amino acids and are therefore

Table 1
Classification of Amino Acids

Essential amino acids	*Conditionally essential amino acids*	*Nonessential amino acids*
Isoleucine[a]	Arginine	Alanine
Leucine[a]	Cysteine (cystine)	Asparagine
Lysine	Glutamine	Aspartic acid
Methionine	Histidine	Glutamic acid
Phenylalanine	Proline	Glycine
Threonine	Tyrosine	Serine
Tryptophan		
Valine[a]		

[a]Branched-chain amino acids

complete sources of protein, whereas plant proteins are missing some of the essential amino acids (i.e., incomplete). Additionally, there are varying levels of quality of protein depending on the amino acid profile of the protein. Complete protein sources that contain larger amounts of essential amino acids generally have higher protein quality.

4.2.1. Whey Protein

Four of the most common types of protein found in protein supplements are whey, casein, soy, and egg (ovalbumin) proteins. Each of these proteins is a complete protein, and all are classified as high quality proteins. Whey protein, derived from milk protein, is currently the most popular source of protein used in nutritional supplements. Whey proteins are available as whey protein concentrates, isolates, and hydrolysates. The primary differences among these forms are the method of processing and small differences in fat and lactose content, amino acid profiles, and ability to preserve glutamine residues. In comparison to other types of protein, whey protein is digested at a faster rate, has better mixing characteristics, and is often perceived as a higher quality protein. Research has indicated that the rapid increase in blood amino acid levels following whey protein ingestion stimulates protein synthesis to a greater degree than casein *(17,18)*. Theoretically, individuals who consume whey protein frequently throughout the day may optimize protein synthesis. In fact, a study by Dangin and associates *(19)* reported that frequent ingestion of a small amount of whey protein served to increase protein synthesis to a greater degree than less frequent ingestion of various proteins. Overall, whey protein is an excellent source of protein to supplement due to its amino acid content (including high branched-chain amino acid content) and its ability to be rapidly absorbed *(20)*.

4.2.2. Casein Protein

Casein, also a milk protein, is often described as a slower-acting protein *(17,19)*. It is considered a slower protein than whey protein because it takes longer to digest and absorb. This is most likely due to fact that casein has a longer transit time in the stomach *(17)*. Although casein stimulates protein synthesis, it does it to a much lesser extent than whey protein *(17)*. Unlike whey, casein helps decrease protein breakdown *(21)*, which has led to the status of casein as having anticatabolic properties. Given the findings that whey protein stimulates protein synthesis and casein helps decrease muscle breakdown, some supplement manufacturers add both whey and casein to their formulations. The effectiveness of combining whey and casein proteins was illustrated in a recent investigation conducted by Kerksick and colleagues *(22)*. In their study, subjects performed a split body part (training the upper body on one day and the lower body on another) resistance training program 4 days a week for 10 weeks. The subjects were given 48 g of carbohydrate *or* 40 g of whey + 8 g of casein *or* 40 g of whey + 5 g of glutamine + 3 g of branched-chain amino acids (BCAAs). After 10 weeks, the group supplemented with combined whey and casein had the largest increase in lean muscle mass.

4.2.3. Soy Protein

Although soy lacks the essential amino acid methionine, it has a relatively high concentration of remaining essential amino acids and is therefore considered a high quality protein. Soy protein is made from soybeans using water or a water–ethanol mixture to extract the protein *(20)*. Soy protein is similar to whey protein in that there is a soy protein concentrate and isolate. Soy contains compounds called isoflavones, which appear to be strong antioxidants and have been implicated in possibly decreasing the risk of developing cardiovascular disease and cancer. In addition to isoflavones, soy proteins contain protease inhibitors. Given these attributes of soy, there is some evidence to suggest that soy may decrease or prevent the exercise-induced damage to muscle seen following a workout *(23)*. At this point, there are few data relative to soy protein ingestion and accretion of lean body mass in conjunction with resistance training; therefore, more research is needed before definitive recommendations can be given.

4.2.4. Egg Protein

Egg protein is also a high quality protein and has the advantage of being a miscible protein (it mixes easily in solution) *(20)*. However, egg protein supplements generally do not taste good and are more expensive than other protein supplements. For these reasons, along with the availability of other high quality proteins such as whey, casein, and soy, egg protein supplementation is not popular among athletes. Despite this, egg protein is still added in small quantities to some meal replacement/protein powders *(20)*.

4.3. Summary

Adequate protein intake consisting of high quality proteins is a prerequisite for the accretion of lean body mass stimulated by a proper resistance training program. Whey, casein, soy, and egg proteins are all high quality proteins and are commonly found in protein supplements marketed to strength-trained athletes. In addition to ingesting the proper amounts and quality of proteins, the timing of protein intake has been a recent area of scientific investigation. A discussion of the importance of this concept, known as "nutrient timing," follows.

5. CARBOHYDRATE–PROTEIN COMBINATIONS

Ingestion of a high quality protein is essential for increasing lean body mass, but equally important is the timing of the protein intake. This category of sports nutrition has been categorized as *nutrient timing*, and there are multiple research studies highlighting the importance of appropriately timing certain meals throughout the day. In summary, the central idea underlying nutrient timing is to time high glycemic carbohydrate and protein ingestion so it encompasses the time frame in which the resistance training bout exerts a hypertrophic stimulus on the trained skeletal muscles. More specifically, stimulated myofibers are "primed" to synthesize protein, but both insulin and amino acid substrate are required to maximize this adaptation in the moments following an acute bout of resistance exercise. This time period following a resistance training session is commonly referred to as the *anabolic window* to emphasize that this time frame has specific anabolic potential.

5.1. Resistance Training in the Absence of Nutritional Intake

Inherent with the term *anabolic window* is the concept of net protein balance. As stated earlier, net protein balance is equal to muscle protein synthesis minus muscle protein breakdown. For skeletal muscle hypertrophy to occur, net protein balance must be positive (synthesis must exceed breakdown). To improve net protein balance, an appropriate stimulus (e.g., resistance training) must be applied to the skeletal muscles. However, when resistance training is performed alone, in the absence of nutritional and supplemental (i.e., protein, carbohydrate) interventions, net protein balance still does not increase to the point of becoming anabolic. Several studies observing the effects of resistance training and acute changes in net protein balance have concluded that net protein balance is improved as a result of the resistance training bout. Although resistance exercise improves the net balance by stimulating muscle protein synthesis, however, nutrient intake is required for the synthesis to exceed the breakdown *(24)*.

As support for this contention, Biolo and colleagues *(1)* assessed rates of protein synthesis and degradation at rest and 3 hours after a resistance training routine in fasted subjects. At 3 hours after exercise, protein synthesis had increased approximately 108% and protein breakdown had increased 51%. Thus, resistance exercise improved the net protein balance by increasing protein synthesis at a greater rate than protein breakdown. Although the net protein balance was improved, it is important to note that it did not improve to the point of becoming positive (anabolic).

Phillips and coworkers *(3)* conducted a similar study in which they recruited two groups of participants (resistance trained and untrained) and had them perform an eccentric-only resistance exercise workout in a fasted state. Rates of protein synthesis and breakdown were measured within 4 hours of completing the resistance training protocol. Following the resistance training bout, muscle protein synthesis rates increased by 118% in the untrained group and by 48% in the resistance trained group. In terms of muscle protein breakdown, there was an increase of 37% in the untrained group and an increase of 15% in the resistance-trained group. Relative to the net protein balance, the resistance training protocol significantly improved this measure in both groups (+37% in the untrained group and +34% in the trained group), but the overall net protein balance was still negative following the bout of resistance training.

Using a larger time frame, this same researcher *(2)* assessed rates of protein synthesis and protein breakdown at rest and at 3, 24, and 48 hours after a resistance training workout in recreationally active (but not previously resistance trained) subjects. Unfortunately, however, the net protein balance was not assessed in the fasted state; rather, each participant ingested food at his own discretion. There was an important nutritional restriction employed: The participants were instructed to eat a meat-free diet during the study (which limited protein intake). In addition, it appears that the 3-hour net protein balance assessment was conducted in the fasted state. Muscle protein synthesis was significantly increased at each time point following the resistance training bout: at 3 hours 112%; at 24 hours 65%; at 48 hours 34%. Muscle protein breakdown was also increased by 31% at 3 hours after exercise and by 18% at 24 hours. Muscle protein breakdown returned to resting levels by 48 hours. One of the novel findings of this study was the observation that muscle protein synthesis was elevated (by 34%) 48 hours after exercise, during which time muscle protein breakdown returned to baseline levels. Despite this finding, at no time point did the net protein balance become positive (likely due to the restrictions on protein intake).

In summary, each of these aforementioned studies indicates that resistance training alone is not enough to elicit positive changes in net protein balance that lead to increases in lean body mass.

5.2. Insulin, Amino Acids, and Protein Synthesis

As stated in the introduction to the chapter, muscle-specific genes must be activated to initiate the process of skeletal muscle hypertrophy. Once these muscle-specific genes are activated, they are copied into messenger RNA (mRNA) which serves as a template for which muscle proteins are then manufactured (translated). Many researchers believe that resistance training acts as the stimulus for activating muscle-specific genes, but once these genes are copied into muscle-specific mRNA transcripts still other factors are needed to convert the muscle-specific mRNA into functional skeletal muscle proteins. Two biological compounds have been shown to be an integral part of this process: insulin and amino acids. In fact, Bolster and coworkers *(25)* stated in a review paper that, "Without question, investigating the singular role of amino acids or insulin in promoting changes in skeletal muscle protein synthesis with resistance exercise is crucial to elucidating mechanisms regulating muscle hypertrophy."

Insulin has several roles relative to improving the net protein balance following resistance exercise, including increasing protein synthesis *(26–28)*, improving the transport of amino acids into skeletal muscle *(27,29,30)*, and decreasing protein breakdown *(30–33)*. Whereas insulin should never be injected (as multiple adverse events are likely to occur) for the purposes of improving net protein balance, insulin can be significantly increased endogenously via the consumption of carbohydrate. As important as insulin concentrations are to anabolic processes, Biolo and Wolfe *(34)* stated that if high levels of insulin are not supported by an exogenous amino acid supply, insulin loses its anabolic capacity in skeletal muscle. This observation has been shared by other investigators as well *(35,36)*.

Relative to protein synthesis, when essential amino acids were ingested after a bout of resistance exercise, the net protein balance was changed from a negative to a positive state *(37)*. Other clinical studies have also demonstrated that the oral ingestion of amino acids are responsible for increasing protein synthesis rates in multiple populations of participants *(38,39)*. Given the importance of insulin and amino acid availability relative to improving net protein balance, ingesting these nutrients simultaneously is recommended. To further this recommendation, by adding a protein source to carbohydrate ingestion it is possible to increase insulin to levels higher than those induced by carbohydrate ingestion alone.

5.3. Importance of Combined Carbohydrate–Protein Supplements and Timing of Ingestion

Carbohydrate (to elevate insulin) and amino acids are needed to maximize positive shifts in net protein balance, and the time course for which they must be present should be considered. To highlight the importance of timing, note that when 10 g of protein, 8 g of carbohydrate, and 3 g of fat were ingested either immediately or 3 hours after exercise, protein synthesis was increased more than threefold with the supplement ingested immediately versus ingestion 3 hours after exercise (with which there was only a 12% increase) *(40)*. In a study by Rasmussen and coworkers *(41)*, subjects were given an amino acid–carbohydrate drink or a placebo following a resistance exercise session. Not surprisingly, the amino acid–carbohydrate drink elicited an anabolic response compared to the placebo. In another study of protein breakdown, Bird and colleagues *(42)* gave subjects one of four supplements after a bout of resistance exercise: 1) carbohydrate beverage; 2) essential amino acids; 3) combination of carbohydrate and amino acids; 4) placebo. The result of this nutritional intervention revealed that protein degradation (as measured by urinary 3-methylhistidine) was elevated at 24 and 48 hours after exercise in the placebo group. Relative to the carbohydrate and amino acid group, protein degradation was unchanged at 24 hours and actually decreased 48 hours after exercise. Given these findings and the data on the aforementioned studies, properly timed carbohydrate–protein/amino acid supplements not only increase protein synthesis but also seem to attenuate protein degradation. Most of the scientific investigations have looked at carbohydrate–protein supplements during the postresistance exercise period; however, one study looked at the difference of ingesting an amino acid–carbohydrate supplement before versus after resistance training *(43)*. The investigators reported that protein synthesis was greater as a result of the preresistance training intake of the amino acid–carbohydrate supplement, most likely due to increased delivery of amino acids to the stimulated skeletal muscle fibers *(43)*.

Most studies have examined the combination of amino acid–carbohydrate supplements in the time frame that encompasses a resistance training session, but not many have investigated intact protein (e.g., whey, casein) supplementation after resistance exercise and their effects on the net protein balance. Tipton et al. *(44)* studied the ingestion of casein and whey proteins and their effects on muscle anabolism after resistance exercise. They concluded that the ingestion of both proteins (whey and casein) after resistance exercise resulted in similar increases in muscle protein net balance, resulting in net muscle protein synthesis, despite different patterns of blood amino acid responses (a quicker response of blood amino acids for the whey protein and a more sustained response for the casein protein). In a similar study, Tipton and coworkers *(45)* questioned if ingestion of whole proteins before exercise would stimulate a superior response to that with ingestion after exercise. The authors reported that the net amino acid balance switched from negative to positive following ingestion of the whey proteins at both time points. In another study, when whey protein was added to an amino acid–carbohydrate supplement, the authors indicated that there seemed to be an extension of the anabolic effect compared to that seen with amino acid–carbohydrate supplements without additional whey protein *(46)*.

5.4. Summary

A proper postworkout supplement designed to increase lean body mass should contain both carbohydrates and protein and be in a liquid form. The reason these carbohydrate–protein supplements should be in liquid form is that liquid meals are more palatable and digestible. In addition, liquid meals have a fast absorption profile compared to that of whole foods, which allows faster insulin secretion and peak plasma amino acid levels—both of which are essential to take advantage of the anabolic window created by the resistance training session. This section has highlighted some of the clinical investigations and the mechanisms as to how appropriately timed ingestion of carbohydrate–protein supplements exert their effects. A more detailed explanation can be found by reading Chapter 13, on dietary meal and nutrient timing.

6. CREATINE

The sports supplement creatine has been the gold standard against which other nutritional supplements are compared. The reason for this prominent position is that creatine improves performance, increases lean body mass, and has repeatedly been shown to be safe when recommended dosages are consumed. Consequently, creatine has become one of the most popular nutritional supplements marketed to athletes over the past decade and a half. In fact, one of the most consistent side effects of creatine supplementation has been weight gain in the form of lean body mass. This increase has been observed in several cohorts including males, females, and the elderly *(47–54)*.

In most of the studies published on creatine supplementation, the typical dosage pattern was divided into two phases: a loading phase and a maintenance phase. A typical loading phase consists of ingesting 20 g of creatine (or 0.3 g/kg body weight) in divided doses four times per day for 2 to 7 days, followed by a maintenance dose of 2 to 5 g daily (or 0.03 g/kg) for several weeks to months at a time *(55)*. Another consideration relative to creatine dosage is to base the amount on an individual's lean body mass. Burke and coworkers *(56)* studied this aspect of creatine supplementation by having subjects ingest creatine at a dosage of 0.1 g/kg of lean body mass (this equates to approximately 8 g of creatine for a 200 pound individual at 15% body fat). Hultman and colleagues *(57)* demonstrated another interesting approach to creatine ingestion. They demonstrated that when creatine was ingested at 3 g/day over an extended training period of at least 4 weeks the skeletal muscle creatine levels rose more slowly, eventually reaching levels similar to those achieved with the loading method.

In summary, a quick way to "creatine load" skeletal muscle requires ingesting 20 g of creatine monohydrate daily for 6 days and then switching to a reduced dosage of 2 g/day *(57)*. If the immediacy of "loading" is not an important consideration, supplementing with 3 g/day for 28 days achieves the same high levels of intramuscular creatine *(57)*.

6.1. Effects on Lean Body Mass

What type of weight gain (in the form of lean body mass) can be expected with this level of creatine supplementation? Many of the studies performed to date indicate that short-term creatine supplementation increases total body mass by approximately 0.7 to 1.6 kg (~1.5–3.5 lb) *(16)*. Longer-term creatine supplementation (~6–8 weeks) in conjunction with resistance training has been shown to increase lean body mass by approximately 2.8 to 3.2 kg (~7 lb) *(58–60)*. Gain in lean body mass has also been observed in women as a result of creatine supplementation. Vandenberghe et al. *(47)* investigated the changes in fat-free mass in females who ingested creatine (20 g/day for the first 4 days followed by 5 g/day for 65 days) in combination with resistance exercise for 10 weeks. The authors reported an increase of 5.7 lb of fat-free mass after 10 weeks of creatine supplementation and resistance exercise. This increase was 60% greater than in the creatine supplementation group compared to the placebo group.

6.2. Physiological Mechanisms for Increasing Lean Body Mass

The exact physiological mechanisms responsible for increasing lean body mass as a result of creatine supplementation remain poorly understood. Early studies investigating creatine supplementation and weight gain led many to the conclusion that increases in body weight were due to water retention. However, several more recent studies suggest that creatine supplementation may help build lean tissue. Volek et al. *(61)* reported that during a 12 week resistance training program, resistance trained males ingesting creatine significantly increased the fat-free mass compared to those ingesting a placebo. Furthermore, it was reported that the subjects given creatine demonstrated significantly greater increases in types I (35% vs. 11%), IIA (36% vs. 15%), and IIAB (35% vs. 6%) muscle fiber cross-sectional areas *(61)*. The percentage increases in cross-sectional area for all fiber types in those subjects ingesting creatine ranged from 29% to 35%—more than twice the increase observed in placebo subjects (6%–15%) *(16)*.

To help elucidate the physiological mechanisms further, Willoughby and Rosene *(62,63)* conducted a series of studies investigating the effects of oral creatine ingestion and the factors involved in gene expression of contractile filaments and myosin heavy-chain protein expression. In the first of these studies, untrained male subjects ingested creatine at 6 g/day or a placebo in conjunction with heavy resistance training for 12 weeks. At the end of the intervention, those ingesting creatine significantly increased their fat-free mass (~7 lb) in comparison with the placebo group (~1 lb). One of the most interesting parameters in this study was the information that was gathered relative to what was occurring at the cellular level of the skeletal muscle. Myofibrillar protein content (a marker of the amount of intracellular protein) was found to be significantly greater in the creatine group than in the placebo group despite the fact that both groups performed identical resistance training programs. More specifically, the authors reported that there were significant increases in the content of two isoforms of myosin heavy-chain protein (the major constituent of contractile skeletal muscle) *(62)*.

In their other study, Willoughby and Rosene *(63)* investigated the effects of ingesting creatine (in conjunction with a resistance training program) on myogenic regulatory factor gene expression. Myogenic regulatory factors (which include Myo-D, myogenin, MRF-4, and Myf5) are proteins that function as transcription activators that regulate gene expression via their binding to DNA, ultimately activating the transcription of muscle-specific genes such as myosin heavy chain, myosin light chain, α-actin, troponin-I, and creatine kinase *(64)*. After 12 weeks of resistance training, the authors reported that the subjects ingesting creatine had significantly greater mRNA expression for myogenin and MRF-4 than the subjects ingesting a placebo. These findings provide an insight into the mechanisms by which creatine supplementation exerts its effects on increasing lean body mass. Taken together, the aforementioned studies seem to indicate that the increases in lean body mass as a result of creatine supplementation are due to augmenting skeletal muscle fiber hypertrophy and not solely water retention.

6.3. Satellite Cell Activity

In addition to increasing muscle fiber cross-sectional areas, myogenic regulatory factors, and specific isoforms of myosin heavy chain, creatine supplementation has been shown to augment an increase in satellite cell number in human skeletal muscle induced by strength training. In addition to muscle-specific transcription and translation, activation of satellite cells is thought to be a major contributing factor to augmenting skeletal muscle hypertrophy. During the process of load-induced muscle hypertrophy, satellite cells are thought to proliferate, differentiate, and then fuse with existing myofibers *(65)*. The way in which satellite cells are thought to be involved in skeletal muscle hypertrophy is summarized in what is termed the *myonuclear domain theory*. This theory suggests that the myonucleus controls the production of mRNA (i.e., transcription) and proteins (i.e., translation) for a finite volume of cytoplasm, such that increases in fiber size must be associated with a proportional increase in myonuclei, which are contributed from the satellite cell populations *(66)*. If this theory is correct, anything that increases satellite cell activity leading to increases in myonuclei sets the stage for increased skeletal muscle hypertrophy.

In a truly original investigation, Olsen and coworkers *(67)* investigated the influence of creatine and protein supplementation on satellite cell frequency and the number of myonuclei in human skeletal muscle during 16 weeks of resistance training. After the 16 weeks of training, all groups in the clinical trial (creatine, protein, and placebo groups) demonstrated significant increases in the proportion of satellite cells. However, only the creatine-supplemented group demonstrated consistent significant increases of myonuclei per fiber. This finding led the authors to conclude that "creatine supplementation in combination with strength training amplifies the training-induced increase in satellite cell number and myonuclei concentration in human skeletal muscle fibers, thereby allowing an enhanced muscle fiber growth in response to strength training" *(67)*. Given this important finding relative to creatine supplementation and satellite cell activity, additional clinical trials investigating this aspect of creatine supplementation are needed.

7. ANABOLIC HORMONE ENHANCERS

Insulin, growth hormone, testosterone, and insulin-like growth factor-1 (IGF-1) are all considered primary anabolic hormones. We have already discussed insulin and its role in translating muscle-specific mRNA into skeletal muscle proteins, and the effects that carbohydrate–amino acid supplements have on increasing insulin levels. The other three anabolic hormones are believed to exert their effects on the cell-signaling properties of skeletal muscle fibers, which ultimately result in muscle-specific gene expression. IGF-1, however, not only acts in this regard (cell signaling) but also acts similarly to insulin in its role of translating muscle-specific mRNA transcripts into functional skeletal muscle proteins (actin, myosin). A further discussion of IGF-1, growth hormone, and testosterone follows.

7.1. Insulin-Like Growth Factor-1

There are three isoforms of IGF-1 in human muscle *(68)*: IGF-1Ea (similar to the type of IGF-1 synthesized in the liver); IGF-1 Eb; and IGF-1Ec (known as mechano growth factor) *(68)*. IGF-1 is produced primarily by the liver as an endocrine hormone and is stimulated by growth hormone release. One of the isoforms of IGF-1, known as mechano growth factor, is detectable only after mechanical stimulation (e.g., resistance training). Skeletal muscle hypertrophy is regulated by at least three major molecular processes: 1) satellite cell activity; 2) gene transcription; and 3) protein translation. Interestingly, IGF-I can influence the activity of all of these mechanisms *(69)*. That being the case, any increases in IGF-1 could significantly increase the potential for skeletal muscle hypertrophy.

In addition to growth hormone release and mechanical stimulation, are there any nutritional or supplemental means that increase endogenous levels of IGF-1? For the most part, the answer is no, but two studies have reported that supplementation with bovine colostrum resulted in increases in serum IGF-I concentration in athletes during training *(70,71)*. However, owing to the relatively acute duration of these studies, lean body mass indices were not measured. Another study that did measure muscle protein balance and strength after 2 weeks of bovine colostrum supplementation *(72)* found that the bovine colostrum had no effect on either of these variables. Given these findings, at this point it is safe to say that there are no sports supplements that effectively increase endogenous IGF-1 levels resulting in changes in lean body mass.

7.2. Growth Hormone

A quick survey of the literature on growth hormone reveals that the hormone does indeed improve body composition by simultaneously increasing lean body mass and decreasing body fat in diseased populations *(73–75)*. However, what is often not mentioned in the marketing campaigns of sports supplements designed to increase growth hormone is the fact that most of these clinical investigations introduced growth hormone into their subjects via subcutaneous injection. Many sports supplements designed to increase endogenous levels of growth hormone are based on studies showing that specific amino acids are able (inconsistently) to increase growth hormone. The main amino acid that has demonstrated potential to increase growth hormone is arginine. As discussed below, arginine is often combined with other compounds to elicit growth hormone release.

It is well documented that the infusion of arginine stimulates growth hormone secretion from the anterior pituitary *(76,77)*. This increase in growth hormone secretion from arginine infusion has been attributed to the suppression of endogenous somatostatin secretion *(76)*. The amounts of arginine infused to elicit the growth hormone response ranged from 12 to 30 g. The clinical investigations observing oral consumption of arginine and its impact on growth hormone release are equivocal. Relative to the practical oral ingestion of arginine, several studies have shown that such supplementation resulted in significant increases in growth hormone secretion.

One such study *(78)* found that oral arginine supplementation of 5 and 9 g resulted in significant growth hormone response in males. Interestingly, 13 g of oral arginine did not increase growth hormone levels and caused gastrointestinal distress in most of the subjects. A common supplemental regimen that has shown promise as a growth hormone enhancer includes the addition of lysine to arginine. Utilizing this combination, Isidori and colleagues *(79)* provided 1.2 g of arginine (as arginine-2-pyrrolidone-5-carboxylate) and 1.2 g of lysine (as lysine hydrochloride) to young males. Plasma growth hormone concentrations increased eightfold at 90 minutes after ingestion. Similarly, Suminski and associates *(80)* reported that the ingestion of arginine and lysine resulted in a 2.7-fold increase in plasma growth hormone concentrations in resistance trained males.

Another compound commonly added to arginine for the purpose of eliciting an increase in growth hormone is aspartate. Besset and colleagues *(81)* gave male subjects arginine aspartate at a dose of 250 mg/kg/day (approximately 17.5 g of arginine aspartate for a 70 kg male) for 1 week. The results indicated that the sleep-related growth hormone peak was about 60% higher after a week of arginine aspartate administration than in the controls. Colombani et al. *(82)* gave 20 male endurance trained athletes 15 g of arginine aspartate (7.5 g in the morning and 7.5 g in the evening) for 14 days before a marathon run. After 31 km had been completed by the runners, plasma growth hormone levels were 40% greater in the arginine aspartate group. At the end of the marathon, plasma growth hormone levels were 8% greater in the supplemented group than in the placebo group.

Not all studies investigating arginine supplementation and growth hormone responses have been favorable. Walberg-Rankin *(83)* gave resistance-trained males ingesting a hypocaloric diet arginine hydrochloride 100 mg/kg/day (approximately 8 g arginine hydrochloride) for a 10-day period. This supplementation protocol did not result in an increase in growth hormone concentration. Another study also reported no increase in plasma growth hormone concentrations when elderly men ingested 3 g of arginine and 3 g lysine for 14 days *(84)*. In yet another study investigating arginine and growth hormone responses, Marcell et al. *(85)* investigated whether oral arginine (5 g) increases growth hormone secretion in young and old people (male and female) at rest and during resistive exercise. The authors concluded that oral arginine supplementation does not increase growth hormone secretion at rest or in combination with resistive exercise.

There are several reasons for the conflicting results in terms of arginine eliciting an increase in growth hormone production. Some of these reasons could be the type of arginine complex, dosages, and delivery methods used and variations in the subjects themselves. It has also been suggested that the growth hormone response to amino acid ingestion may be reduced in individuals who are exercise trained *(55)*.

Even if certain amino acids do increase growth hormone levels (a statement not supported by all investigations), it does not necessarily lead to the conclusion that they increase lean body mass. In a scientific review on this subject, Chromiak and Antonio *(55)* stated: “There is no evidence based on properly conducted, rigorous scientific studies that oral supplementation of specific amino acids induces growth hormone that, in conjunction with resistance training, increases muscle mass and strength to a greater extent than resistance training alone.” At this point, it appears as if specific amino acids, even if they do elicit an increase in growth hormone, do not increase lean body mass via this mechanism.

7.3. Testosterone

Although each of the anabolic hormones (testosterone, growth hormone, insulin, IGF-1) is required to stimulate maximum levels of skeletal muscle hypertrophy, testosterone may be the most anabolic. It is important to recognize that not all of the testosterone in the blood is bioavailable; rather, most of it is bound to proteins such as sex hormone-binding globulin (SHBG) or other carrier proteins. Testosterone that is not bound is referred to as "free" or "bioavailable" testosterone; and it is able to bind to the androgen receptor and exert its anabolic signaling. This is an important distinction because as one attempts to increase testosterone levels (via testosterone-enhancing supplements) in the body, it is only the bioavailable testosterone that exerts anabolic actions. Another important consideration is the avoidance of increasing SHBG to a greater extent than total testosterone increases, which would result in an environment in which there is less bioavailable testosterone present. Therefore, when investigating sports supplements designed to increase testosterone, each of these factors must be considered. Currently, there are a few sports supplements that claim to increase testosterone levels: ZMA, *Tribulus terrestris*, and aromatase inhibitors.

7.3.1. ZMA

The primary ingredients in ZMA supplements are zinc monomethionine aspartate, magnesium aspartate, and vitamin B_6. Zinc and magnesium deficiencies as well as urine and sweat losses of these minerals have been observed in athletes and individuals who are physically active *(86–90)*. Relative to testosterone, there have been two well designed studies investigating the effects of ZMA supplementation and its effects on testosterone levels, with the studies reporting contradictory results *(91,92)*.

The first of these studies gave collegiate football players ZMA (30 mg zinc monomethionine aspartate + 450 mg magnesium aspartate + 10.5 mg of vitamin B_6) over the course of their spring practice season (approximately 8 weeks) *(91)*. Total testosterone and, more importantly, free testosterone were significantly elevated as a result of the ZMA supplementation compared to that of the placebo group. This study is consistently cited as proof of the effectiveness of ZMA to elevate testosterone levels. In the other study *(92)*, researchers gave resistance trained males a ZMA supplement (main ingredients consisting of 30 mg zinc monomethionine aspartate + 450 mg magnesium aspartate + and 11 mg of vitamin B_6) and found no such increases in either total or free testosterone. This investigation *(92)* also assessed changes in the fat-free mass and several strength and performance variables. No significant differences were observed in relation to these variables in subjects taking ZMA. The discrepancies concerning these two studies may be explained by deficiencies of these minerals. Given the role that zinc deficiency plays relative to androgen metabolism and interaction with steroid receptors *(93)*, when there are deficiencies of this mineral, testosterone production may suffer. In the study showing increases in testosterone levels *(91)*, there were observed depletions of both zinc and magnesium in the placebo group over the course of the study. Therefore, the increased testosterone levels could have been attributed to impaired nutritional status rather than a pharmacological effect. Obviously, more research is needed on supplemental ZMA before any concrete recommendations can be made relative to testosterone responses.

7.3.2. *Tribulus terrestris*

Tribulus terrestris is often marketed as a testosterone-boosting sports supplement. There are relatively few, if any, scientific studies to substantiate these claims. In fact, one clinical investigation demonstrated that *Tribulus terrestris* exerts no effect on increasing testosterone levels *(94)*. In this study, healthy men were instructed to supplement with *Tribulus terrestris* for a 4-week period after which serum levels of testosterone and luteinizing hormone were measured at 1, 3, 10, 17, and 24 days after supplementation. *Tribulus terrestris* supplementation did not increase the levels of either testosterone or luteinizing hormone. Given the unsubstantiated claims of *Tribulus terrestris* relative to increasing testosterone levels, supplemental *Tribulus* is not recommended.

7.3.3. Aromatase Inhibitors

Aromatase inhibitors exert their effects by inhibiting the action of the enzyme aromatase, which converts androgens to estrogens by a process called aromatization. Aromatase inhibitor supplements claim to suppress estrogen levels and increase endogenous testosterone levels. In the only published study investigating aromatase inhibitor supplements, Willoughby and colleagues *(95)* instructed their male subjects to ingest an aromatase inhibitor supplement (containing hydroxyandrost-4-ene-6,17 dioxo-3-THP ether and 3,17-diketo-androst-1,4,6-triene) at 72 mg/day for an 8-week period. At the end of the 8 weeks there was a 3-week washout period. Multiple anabolic hormones were assayed during the duration of the study, including total testosterone and free testosterone. In addition, body composition was assessed during the investigation in which the participants were instructed to maintain their normal resistance training programs. There were significant increases in both total and free testosterone levels compared to those with placebos, with the total testosterone having an average increase of 283% and free testosterone an average of 625%. The aromatase inhibitor supplement had also elicited a 3.5% decrease in fat mass in the aromatase inhibitor group at the end of the 8-week period. After the 3-week washout period, total and free testosterone levels decreased to the presupplementation values. Finally, the aforementioned supplementation appeared to be safe and well tolerated by the study participants as measured by blood and urinary clinical safety markers. Although this study appears to support aromatase inhibitor supplementation for the purpose of increasing endogenous testosterone levels, additional studies are needed to replicate these findings. In summary, it appears that of all the nutritional supplements designed to increase testosterone levels aromatase inhibitor supplementation is the most scientifically valid option.

8. ANTICATABOLIC SUPPLEMENTS

Because the net protein balance is equal to muscle protein synthesis minus muscle protein breakdown, eliciting increases in lean body mass can be achieved not only by increasing protein synthesis but also by decreasing protein breakdown (catabolism). Hence, a number of sports supplements are marketed for that endeavor, including glutamine, cortisol inhibitors, β-hydroxyl-β-methylbutyrate (HMB), and α-ketoisocaproic acid. In addition to these specific sports supplements, insulin has been shown repeatedly to suppress protein breakdown *(30,31,96,97)*. Hence, carbohydrate (or carbohydrate + protein) taken after resistance exercise (a period when protein breakdown is elevated) for the purpose of increasing insulin secretion is a recommended practice to suppress protein breakdown *(42)*. Other purported anticatabolic supplements are discussed below.

8.1. α-Ketoisocaproic Acid

α-Ketoisocaproic acid (KIC) is the keto acid of the BCAA leucine. Despite many claims of KIC and its anticatabolic properties, there is only one peer-reviewed study in humans that has investigated the inclusion of KIC (along with HMB) on a specific marker of muscle damage (creatine kinase). When non-resistance-trained males ingested 3 g of HMB and 0.3 g of KIC daily for 14 days prior to a resistance training session, it was reported that the HMB–KIC supplementation attenuated the creatine kinase response compared to that seen with the placebo *(98)*. Although this may be an important finding, creatine kinase is not a direct measure of protein breakdown. Also, the extent to which HMB or KIC alone affected this attenuation of creatine kinase cannot be determined. Another study that is commonly cited as evidence for KIC supplementation and its ability to prevent proteolysis was conducted on isolated rat diaphragm skeletal muscle *(99)*. In this venue, one should be cautious of overextrapolating from rodent data to the human condition. At this point, there are not enough data to conclude that KIC supplementation alone is an effective anticatabolic supplement.

8.2. β-Hydroxy-β-Methylbutyrate

β-Hydroxy-β-methylbutyrate is a metabolite of the BCAA leucine and is often associated with anticatabolic potential. The original research study to highlight HMB's anticatabolic potential was conducted by Nissen and coworkers *(100)*. In this study, untrained subjects ingested one of three levels of HMB (0, 1.5, or 3.0 g/day) and two protein levels (117 or 175 g/day) and resistance trained 3 days per week for 3 weeks. Other markers of muscle damage were assessed, and protein breakdown was assessed by measuring urinary 3-methylhistidine (3-MH). After the first week of the resistance training protocol, urinary 3-MH was increased by 94% in the control group and by 85% and 50% in individuals ingesting 1.5 and 3.0 g of HMB per day, respectively. During the second week of the study, 3-MH levels were still elevated by 27% in the control group but were 4% and 15% below basal levels for the groups on HMB 1.5 and 3.0 g/day. Interestingly, 3-MH measures at the end of the third week of resistance training were not significantly different among the groups *(100)*. Other studies demonstrating an anticatabolic effect or suppressing muscle damage have supported the finding of this study *(98,101)*. A study conducted by van Someren and coworkers *(98)* instructed their male subjects to ingest 3 g of HMB in addition to 0.3 g KIC daily for 14 days prior to performing a single bout of eccentrically biased resistance exercise. This supplemental intervention that included HMB resulted in a significant reduction in the plasma markers of muscle damage.

Although HMB supplementation may suppress protein breakdown and markers of muscle damage, the main question is if this anticatabolic effect leads to gains in lean body mass. The scientific literature on this topic is divided. In a second arm to the study conducted by Nissen and colleagues *(100)*, male subjects ingested 3 g of HMB or a placebo for 7 weeks in conjunction with resistance training 6 days per week. In this study, the fat-free mass increased in the HMB-supplemented group at various times throughout the investigative period but not at the conclusion of the study. Other studies have also reported evidence for HMB supplementation (~3 g/day) relative to increasing lean body mass *(102,103)*. In addition, a meta-analysis conducted by Nissen and Sharp *(104)* stated that only HMB and one other sports supplement (creatine) were found to increase lean body mass significantly.

Not all studies agree with the findings that HMB increases lean body mass, however *(105–107)*. Each of the studies showing no effect of HMB on lean body mass accretion also supplemented their subjects with approximately the same amount of HMB as the studies that demonstrated increases in lean body mass. Although not conclusive, it appears that HMB supplementation does suppress protein breakdown, ultimately leading to increased lean body mass in some individuals. Following carbohydrate supplementation (for the purpose of secreting insulin), HMB is the next best anticatabolic sports supplement.

8.3. Glutamine

Another sports supplement commonly marketed as an anticatabolic agent is the amino acid glutamine. Glutamine is the most abundant free amino acid in plasma and skeletal muscle and accounts for more than half of the total intramuscular free amino acid pool *(108)*. The rationale for glutamine's anticatabolic effects is the fact that it is one of the major fuels used by the gut, resulting in a high cellular turnover of glutamine in the gut (intestinal mucosal cells). This high turnover may result in the supply of amino acids (glutamine) to the cells of the gastrointestinal tract at the expense of skeletal muscle protein. By providing supplemental glutamine to the gut, it theoretically spares the glutamine that is available in skeletal muscle and in this way serves as an anticatabolic agent. One clinical investigation that gave supplemental glutamine to individuals engaging in resistance exercise did not demonstrate an anticatabolic potential or result in increases in lean body mass *(109)*. Other studies that have demonstrated anticatabolic potential for glutamine have used critically ill subjects or subjects who underwent surgery *(110, 111)*. Despite a valid theoretical rationale for glutamine supplementation, at this point there are no scientific data demonstrating that glutamine supplementation suppresses protein breakdown in resistance trained individuals.

9. NITRIC OXIDE BOOSTERS

One of the more recent developments in sports supplements has been the introduction of supplements intended to increase nitric oxide production. Relative to biological processes in humans, nitric oxide is synthesized in cells by nitric oxide synthase. One of nitric oxide's primary physiological functions is to relax smooth muscle, and hence it is one of the body's major regulators of blood flow, especially during exercise. Kingwell *(112)* indicated that nitric oxide potentially affects metabolic control during exercise via multiple mechanisms, including:

- Elevation in skeletal muscle and cardiac blood flow and increased delivery of oxygen, substrates, and regulatory hormones (e.g., insulin)
- Preservation of intracellular skeletal muscle energy stores by promoting glucose uptake, inhibiting glycolysis, mitochondrial respiration, and phosphocreatine breakdown

Together, these actions of nitric oxide on blood flow and substrate utilization appear to be directed toward protection from ischemia *(112)*. Also, if adequate amounts of oxygen and substrate are supplied to the skeletal muscle undergoing mechanical stress, the possibility of extending the total workload on each set of a resistance training bout may lead to greater stimulus for muscle fiber hypertrophy. The aforementioned observation, in conjunction with some earlier studies showing nitric oxide as an integral compound relative to improving skeletal muscle's force production and maximal power output *(113,114)*, has provided a rationale for investigating ways to increase endogenous nitric oxide production.

In every sports supplement claiming to augment endogenous nitric oxide production, the amino acid arginine is included in the list of ingredients. This is due to the fact that arginine serves as a precursor for the biosynthesis of nitric oxide *(115)*. In fact, arginine is the only endogenous nitrogen-containing substrate of nitric oxide synthase and thus governs production of nitric oxide. To date, only one study has investigated the effects of an arginine-containing sports supplement aiming at augmenting endogenous nitric oxide production *(116)*. Although this study was well designed, it should be noted that nitric oxide production was not assessed in the clinical investigation. The study investigated the effects of ingesting 12 g of arginine α-ketoglutarate in conjunction with a periodized resistance training program over a period of 8 weeks. Although there was some improvement in some exercise performance variables, the investigators did not observe any increases in lean body mass. Specifically, those ingesting arginine α-ketoglutarate had a significant increase in upper body strength (as measured by the bench press) compared to the subjects ingesting a placebo. In fact, those ingesting the arginine α-ketoglutarate increased their bench press by 19 lb versus an increase of approximately 6 lb in the placebo group. Additionally, the arginine α-ketoglutarate group significantly increased their peak power output (as measured by a 30-second cycle sprint test) in comparison to the placebo group. Whether these improvements in exercise performance can be associated with increases in nitric oxide production is not known, but the fact remains that there were no increases relative to lean body mass observed in this investigation. At this point, there is a theoretical rationale that augmenting nitric oxide production may lead to more intense training and ultimately greater skeletal muscle hypertrophy. However, because clinical investigations have not demonstrated this, it is premature to state emphatically that sports supplements designed to increase nitric oxide lead to greater gains in lean body mass.

10. CONCLUSION

Increasing lean body mass is a goal of many athletes, recreational weight trainers, and those who wish to improve their body composition. When choosing a dietary supplement to augment increases in lean body mass, it is important to consider the way in which the supplement contributes to the highly regulated process of skeletal muscle hypertrophy. The science of sports supplements is relatively new, although certain sports supplements (protein, creatine) have been scientifically investigated and have repeatedly demonstrated their ability to increase lean body mass. Other sports supplements (e.g., anticatabolic agents, anabolic hormone enhancers, nitric oxide boosters) require more rigorous scientific investigation before they can be deemed effective (or not).

REFERENCES

1. Biolo G, Maggi SP, Williams BD, Tipton KD, Wolfe RR. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. Am J Physiol 1995;268(Pt 1):E514–E20.
2. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol 1997;273(Pt 1):E99–E107.
3. Phillips SM, Tipton KD, Ferrando AA, Wolfe RR. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. Am J Physiol 1999;276(Pt 1):E118–E124.
4. Wagenmakers AJ. Tracers to investigate protein and amino acid metabolism in human subjects. Proc Nutr Soc 1999;58:987–1000.
5. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002;102:1621–1630.
6. American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada. Joint position statement: nutrition and athletic performance. Med Sci Sports Exerc 2000;32:2130–2145.
7. Tarnopolsky M. Protein requirements for endurance athletes. Nutrition 2004;20:662–668.

8. Forslund AH, El-Khoury AE, Olsson RM, Sjodin AM, Hambraeus L, Young VR. Effect of protein intake and physical activity on 24-h pattern and rate of macronutrient utilization. Am J Physiol 1999;276(Pt 1):E964–E976.
9. Meredith CN, Zackin MJ, Frontera WR, Evans WJ. Dietary protein requirements and body protein metabolism in endurance-trained men. J Appl Physiol 1989;66:2850–2856.
10. Phillips SM, Atkinson SA, Tarnopolsky MA, MacDougall JD. Gender differences in leucine kinetics and nitrogen balance in endurance athletes. J Appl Physiol 1993;75:2134–2141.
11. Lamont LS, Patel DG, Kalhan SC. Leucine kinetics in endurance-trained humans. J Appl Physiol 1990;69:1–6.
12. Friedman JE, Lemon PW. Effect of chronic endurance exercise on retention of dietary protein. Int J Sports Med 1989;10:118–123.
13. Tarnopolsky MA, Atkinson SA, MacDougall JD, Chesley A, Phillips S, Schwarcz HP. Evaluation of protein requirements for trained strength athletes. J Appl Physiol 1992;73:1986–1995.
14. Lemon PW, Tarnopolsky MA, MacDougall JD, Atkinson SA. Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders. J Appl Physiol 1992;73:767–775.
15. Lemon PW. Protein and amino acid needs of the strength athlete. Int J Sport Nutr 1991;1:127–145.
16. Antonio J, Stout J (eds). Sports Supplements. Lippincott Williams & Wilkins, Philadelphia, 2001.
17. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci U S A 1997;94:14930–14935.
18. Fruhbeck G. Protein metabolism: slow and fast dietary proteins. Nature 1998;391:843, 845.
19. Dangin M, Boirie Y, Garcia-Rodenas C, et al. The digestion rate of protein is an independent regulating factor of postprandial protein retention. Am J Physiol Endocrinol Metab 2001;280:E340–E348.
20. Driskell J, Wolinsky I (eds) Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition. CRC Press, Boca Raton, FL, 2000.
21. Demling RH, DeSanti L. Effect of a hypocaloric diet, increased protein intake and resistance training on lean mass gains and fat mass loss in overweight police officers. Ann Nutr Metab 2000;44:21–29.
22. Kerksick CM, Rasmussen CJ, Lancaster SL, et al. The effects of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training. J Strength Cond Res 2006;20:643–653.
23. Nikawa T, Ikemoto M, Sakai T, et al. Effects of a soy protein diet on exercise-induced muscle protein catabolism in rats. Nutrition 2002;18:490–495.
24. Wolfe RR. Effects of amino acid intake on anabolic processes. Can J Appl Physiol 2001;26(Suppl):S220–S227.
25. Bolster DR, Jefferson LS, Kimball SR. Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signalling. Proc Nutr Soc 2004;63:351–356.

26. Kimball SR, Jurasinski CV, Lawrence JC Jr, Jefferson LS. Insulin stimulates protein synthesis in skeletal muscle by enhancing the association of eIF-4E and eIF-4 G. Am J Physiol 1997;272(Pt 1):C754–C759.
27. Biolo G, Declan Fleming RY, Wolfe RR. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. J Clin Invest 1995;95:811–819.
28. Hillier TA, Fryburg DA, Jahn LA, Barrett EJ. Extreme hyperinsulinemia unmasks insulin's effect to stimulate protein synthesis in the human forearm. Am J Physiol 1998;274(Pt 1):E1067–E1074.
29. Gore DC, Wolf SE, Sanford AP, Herndon DN, Wolfe RR. Extremity hyperinsulinemia stimulates muscle protein synthesis in severely injured patients. Am J Physiol Endocrinol Metab 2004;286:E529–E534.
30. Biolo G, Williams BD, Fleming RY, Wolfe RR. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. Diabetes 1999;48:949–957.
31. Gelfand RA, Barrett EJ. Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. J Clin Invest 1987;80:1–6.
32. Heslin MJ, Newman E, Wolf RF, Pisters PW, Brennan MF. Effect of hyperinsulinemia on whole body and skeletal muscle leucine carbon kinetics in humans. Am J Physiol 1992;262(Pt 1):E911–E918.
33. Denne SC, Liechty EA, Liu YM, Brechtel G, Baron AD. Proteolysis in skeletal muscle and whole body in response to euglycemic hyperinsulinemia in normal adults. Am J Physiol 1991;261(Pt 1):E809–E814.
34. Biolo G, Wolfe RR. Insulin action on protein metabolism. Baillieres Clin Endocrinol Metab 1993;7:989–1005.
35. Bell JA, Fujita S, Volpi E, Cadenas JG, Rasmussen BB. Short-term insulin and nutritional energy provision do not stimulate muscle protein synthesis if blood amino acid availability decreases. Am J Physiol Endocrinol Metab 2005;289:E999–E1006.
36. Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. Am J Physiol Endocrinol Metab 2006;291:E745–E754.
37. Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol 1999;276(Pt 1):E628–E634.
38. Paddon-Jones D, Sheffield-Moore M, Zhang XJ, et al. Amino acid ingestion improves muscle protein synthesis in the young and elderly. Am J Physiol Endocrinol Metab 2004;286:E321–E328.
39. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 2003;78:250–258.
40. Levenhagen DK, Gresham JD, Carlson MG, Maron DJ, Borel MJ, Flakoll PJ. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. Am J Physiol Endocrinol Metab 2001;280:E982–E993.

41. Rasmussen BB, Tipton KD, Miller SL, Wolf SE, Wolfe RR. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol 2000;88:386–392.
42. Bird SP, Tarpenning KM, Marino FE. Liquid carbohydrate/essential amino acid ingestion during a short-term bout of resistance exercise suppresses myofibrillar protein degradation. Metabolism 2006;55:570–577.
43. Tipton KD, Rasmussen BB, Miller SL, et al. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. Am J Physiol Endocrinol Metab 2001;281:E197–E206.
44. Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP, Wolfe RR. Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. Med Sci Sports Exerc 2004;36:2073–2081.
45. Tipton KD, Elliott TA, Cree MG, Aarsland AA, Sanford AP, Wolfe RR. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. Am J Physiol Endocrinol Metab 2007;292:E71–E76.
46. Borsheim E, Aarsland A, Wolfe RR. Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise. Int J Sport Nutr Exerc Metab 2004;14:255–271.
47. Vandenberghe K, Goris M, Van Hecke P, Van Leemputte M, Vangerven L, Hespel P. Long-term creatine intake is beneficial to muscle performance during resistance training. J Appl Physiol 1997;83:2055–2063.
48. Kelly V, Jenkins D. Effect of oral creatine supplementation on near-maximal strength and repeated sets of high intensity bench press exercise. J Strength Cond Res 1998;12:109–115.
49. Van Loon LJ, Oosterlaar AM, Hartgens F, Hesselink MK, Snow RJ, Wagenmakers AJ. Effects of creatine loading and prolonged creatine supplementation on body composition, fuel selection, sprint and endurance performance in humans. Clin Sci (Lond) 2003;104:153–162.
50. Kreider RB, Ferreira M, Wilson M, et al. Effects of creatine supplementation on body composition, strength, and sprint performance. Med Sci Sports Exerc 1998;30:73–82.
51. Branch JD. Effect of creatine supplementation on body composition and performance: a meta-analysis. Int J Sport Nutr Exerc Metab 2003;13:198–226.
52. Gotshalk LA, Volek JS, Staron RS, Denegar CR, Hagerman FC, Kraemer WJ. Creatine supplementation improves muscular performance in older men. Med Sci Sports Exerc 2002;34:537–543.
53. Brose A, Parise G, Tarnopolsky MA. Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. J Gerontol A Biol Sci Med Sci 2003; 58:11–19.
54. Chrusch MJ, Chilibeck PD, Chad KE, Davison KS, Burke DG. Creatine supplementation combined with resistance training in older men. Med Sci Sports Exerc 2001;33:2111–2117.
55. Chromiak JA, Antonio J. Use of amino acids as growth hormone-releasing agents by athletes. Nutrition 2002;18:657–661.

56. Burke DG, Smith-Palmer T, Holt LE, Head B, Chilibeck PD. The effect of 7 days of creatine supplementation on 24-hour urinary creatine excretion. J Strength Cond Res 2001;15:59–62.
57. Hultman E, Soderlund K, Timmons JA, Cederblad G, Greenhaff PL. Muscle creatine loading in men. J Appl Physiol 1996;81:232–237.
58. Stout J, Eckerson J, Noonan D. Effects of 8 weeks of creatine supplementation on exercise performance and fat-free weight in football players during training. Nutr Res 1999;19:217–225.
59. Earnest CP, Snell PG, Rodriguez R, Almada AL, Mitchell TL. The effect of creatine monohydrate ingestion on anaerobic power indices, muscular strength and body composition. Acta Physiol Scand 1995;153:207–209.
60. Kreider RB, Klesges R, Harmon K, et al. Effects of ingesting supplements designed to promote lean tissue accretion on body composition during resistance training. Int J Sport Nutr 1996;6:234–246.
61. Volek JS, Duncan ND, Mazzetti SA, et al. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. Med Sci Sports Exerc 1999;31:1147–1156.
62. Willoughby DS, Rosene J. Effects of oral creatine and resistance training on myosin heavy chain expression. Med Sci Sports Exerc 2001;33:1674–1681.
63. Willoughby DS, Rosene JM. Effects of oral creatine and resistance training on myogenic regulatory factor expression. Med Sci Sports Exerc 2003;35:923–929.
64. Lowe DA, Lund T, Alway SE. Hypertrophy-stimulated myogenic regulatory factor mRNA increases are attenuated in fast muscle of aged quails. Am J Physiol 1998;275(Pt 1):C155–C162.
65. Schultz E, McCormick KM. Skeletal muscle satellite cells. Rev Physiol Biochem Pharmacol 1994;123:213–257.
66. Hawke TJ. Muscle stem cells and exercise training. Exerc Sport Sci Rev 2005;33:63–68.
67. Olsen S, Aagaard P, Kadi F, et al. Creatine supplementation augments the increase in satellite cell and myonuclei number in human skeletal muscle induced by strength training. J Physiol 2006;573(Pt 2):525–534.
68. Hameed M, Orrell RW, Cobbold M, Goldspink G, Harridge SD. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. J Physiol 2003;547(Pt 1):247–254.
69. Spangenburg EE. IGF-I isoforms and ageing skeletal muscle: an ‘unresponsive’ hypertrophy agent? J Physiol 2003;547(Pt 1):2.
70. Mero A, Miikkulainen H, Riski J, Pakkanen R, Aalto J, Takala T. Effects of bovine colostrum supplementation on serum IGF-I, IgG, hormone, and saliva IgA during training. J Appl Physiol 1997;83:1144–1151.
71. Mero A, Kahkonen J, Nykanen T, et al. IGF-I, IgA, and IgG responses to bovine colostrum supplementation during training. J Appl Physiol 2002;93:732–739.
72. Mero A, Nykanen T, Keinanen O, et al. Protein metabolism and strength performance after bovine colostrum supplementation. Amino Acids 2005;28:327–335.
73. Fernholm R, Bramnert M, Hagg E, et al. Growth hormone replacement therapy improves body composition and increases bone metabolism in elderly patients with pituitary disease. J Clin Endocrinol Metab 2000;85:4104–4112.

74. Thoren M, Hilding A, Baxter RC, Degerblad M, Wivall-Helleryd IL, Hall K. Serum insulin-like growth factor I (IGF-I), IGF-binding protein-1 and -3, and the acid-labile subunit as serum markers of body composition during growth hormone (GH) therapy in adults with GH deficiency. J Clin Endocrinol Metab 1997;82:223–228.
75. Ahmad AM, Hopkins MT, Thomas J, Ibrahim H, Fraser WD, Vora JP. Body composition and quality of life in adults with growth hormone deficiency; effects of low-dose growth hormone replacement. Clin Endocrinol (Oxf) 2001;54:709–717.
76. Alba-Roth J, Muller OA, Schopohl J, von Werder K. Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. J Clin Endocrinol Metab 1988;67:1186–1189.
77. Merimee TJ, Rabinowitz D, Riggs L, Burgess JA, Rimoin DL, McKusick VA. Plasma growth hormone after arginine infusion: clinical experiences. N Engl J Med 1967;276:434–439.
78. Collier SR, Casey DP, Kanaley JA. Growth hormone responses to varying doses of oral arginine. Growth Horm IGF Res 2005;15:136–139.
79. Isidori A, Lo Monaco A, Cappa M. A study of growth hormone release in man after oral administration of amino acids. Curr Med Res Opin 1981;7:475–481.
80. Suminski RR, Robertson RJ, Goss FL, et al. Acute effect of amino acid ingestion and resistance exercise on plasma growth hormone concentration in young men. Int J Sport Nutr 1997;7:48–60.
81. Besset A, Bonardet A, Rondouin G, Descomps B, Passouant P. Increase in sleep related GH and Prl secretion after chronic arginine aspartate administration in man. Acta Endocrinol (Copenh) 1982;99:18–23.
82. Colombani PC, Bitzi R, Frey-Rindova P, et al. Chronic arginine aspartate supplementation in runners reduces total plasma amino acid level at rest and during a marathon run. Eur J Nutr 1999;38:263–270.
83. Walberg-Rankin J, Hawkins C, Fild D, Sebolt D. The effect of oral arginine during energy restriction in male weight trainers. J Strength Cond Res 1994;8:170–177.
84. Corpas E, Blackman MR, Roberson R, Scholfield D, Harman SM. Oral arginine-lysine does not increase growth hormone or insulin-like growth factor-I in old men. J Gerontol 1993;48:M128–M133.
85. Marcell TJ, Taaffe DR, Hawkins SA, et al. Oral arginine does not stimulate basal or augment exercise-induced GH secretion in either young or old adults. J Gerontol A Biol Sci Med Sci 1999;54:M395–M399.
86. Lukaski HC. Micronutrients (magnesium, zinc, and copper): are mineral supplements needed for athletes? Int J Sport Nutr 1995;5(Suppl):S74–S83.
87. Lukaski HC. Magnesium, zinc, and chromium nutriture and physical activity. Am J Clin Nutr 2000;72(Suppl):585S–593S.
88. Kikukawa A, Kobayashi A. Changes in urinary zinc and copper with strenuous physical exercise. Aviat Space Environ Med 2002;73:991–995.
89. Nielsen FH, Lukaski HC. Update on the relationship between magnesium and exercise. Magnes Res 2006;19:180–189.
90. Buchman AL, Keen C, Commisso J, et al. The effect of a marathon run on plasma and urine mineral and metal concentrations. J Am Coll Nutr 1998;17:124–127.

91. Brilla L, Conte V. Effects of a novel zinc-magnesium formulation on hormones and strength. J Exerc Physiol Online 2000;3:26–36.
92. Wilborn C, Kerksick CM, Campbell B, et al. Effects of zinc magnesium aspartate (ZMA) supplementation on training adaptations and markers of anabolism and catabolism. J Int Soc Sports Nutr 2004;1:12–20.
93. Om AS, Chung KW. Dietary zinc deficiency alters 5 alpha-reduction and aromatization of testosterone and androgen and estrogen receptors in rat liver. J Nutr 1996;126:842–848.
94. Neychev VK, Mitev VI. The aphrodisiac herb Tribulus terrestris does not influence the androgen production in young men. J Ethnopharmacol 2005;101:319–323.
95. Willoughby DS, Wilborn C, Taylor L, Campbell B. Eight weeks of aromatase inhibition using the nutritional supplement Novedex XT: effects in young, eugonadal men. Int J Sport Nutr Exerc Metab 2007;17:92–108.
96. Fryburg DA, Barrett EJ, Louard RJ, Gelfand RA. Effect of starvation on human muscle protein metabolism and its response to insulin. Am J Physiol 1990;259(Pt 1):E477–E482.
97. Fryburg DA, Louard RJ, Gerow KE, Gelfand RA, Barrett EJ. Growth hormone stimulates skeletal muscle protein synthesis and antagonizes insulin's antiproteolytic action in humans. Diabetes 1992;41:424–429.
98. Van Someren KA, Edwards AJ, Howatson G. Supplementation with beta-hydroxy-beta-methylbutyrate (HMB) and alpha-ketoisocaproic acid (KIC) reduces signs and symptoms of exercise-induced muscle damage in man. Int J Sport Nutr Exerc Metab 2005;15:413–424.
99. Tischler M, Goldberg A. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? J Biol Chem 1982;257:1613–1621.
100. Nissen S, Sharp R, Ray M, et al. Effect of leucine metabolite beta-hydroxy-beta-methylbutyrate on muscle metabolism during resistance-exercise training. J Appl Physiol 1996;81:2095–2104.
101. Knitter AE, Panton L, Rathmacher JA, Petersen A, Sharp R. Effects of beta-hydroxy-beta-methylbutyrate on muscle damage after a prolonged run. J Appl Physiol 2000;89:1340–1344.
102. Jowko E, Ostaszewski P, Jank M, et al. Creatine and beta-hydroxy-beta-methylbutyrate (HMB) additively increase lean body mass and muscle strength during a weight-training program. Nutrition 2001;17:558–566.
103. Gallagher PM, Carrithers JA, Godard MP, Schulze KE, Trappe SW. Beta-hydroxy-beta-methylbutyrate ingestion. I. Effects on strength and fat free mass. Med Sci Sports Exerc 2000;32:2109–2115.
104. Nissen SL, Sharp RL. Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis. J Appl Physiol 2003;94:651–659.
105. Kreider RB, Ferreira M, Wilson M, Almada AL. Effects of calcium beta-hydroxy-beta-methylbutyrate (HMB) supplementation during resistance-training on markers of catabolism, body composition and strength. Int J Sports Med 1999;20:503–509.

106. Slater G, Jenkins D, Logan P, et al. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation does not affect changes in strength or body composition during resistance training in trained men. Int J Sport Nutr Exerc Metab 2001;11:384–396.
107. Vukovich MD, Stubbs NB, Bohlken RM. Body composition in 70-year-old adults responds to dietary beta-hydroxy-beta-methylbutyrate similarly to that of young adults. J Nutr 2001;131:2049–2052.
108. Curthoys NP, Watford M. Regulation of glutaminase activity and glutamine metabolism. Annu Rev Nutr 1995;15:133–159.
109. Candow DG, Chilibeck PD, Burke DG, Davison KS, Smith-Palmer T. Effect of glutamine supplementation combined with resistance training in young adults. Eur J Appl Physiol 2001;86:142–149.
110. Hammarqvist F, Wernerman J, Ali R, von der Decken A, Vinnars E. Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis, and improves nitrogen balance. Ann Surg 1989;209:455–461.
111. Vinnars E, Hammarqvist F, von der Decken A, Wernerman J. Role of glutamine and its analogs in posttraumatic muscle protein and amino acid metabolism. JPEN J Parenter Enteral Nutr 1990;14(Suppl):125S–129S.
112. Kingwell BA. Nitric oxide-mediated metabolic regulation during exercise: effects of training in health and cardiovascular disease. FASEB J 2000;14:1685–1696.
113. Morrison RJ, Miller CC 3rd, Reid MB. Nitric oxide effects on shortening velocity and power production in the rat diaphragm. J Appl Physiol 1996;80:1065–1069.
114. Morrison RJ, Miller CC 3rd, Reid MB. Nitric oxide effects on force-velocity characteristics of the rat diaphragm. Comp Biochem Physiol A Mol Integr Physiol 1998;119:203–209.
115. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002–2012.
116. Campbell B, Roberts M, Kerksick C, et al. Pharmacokinetics, safety, and effects on exercise performance of L-arginine alpha-ketoglutarate in trained adult men. Nutrition 2006;22:872–881.