

SCIENTIFIC SUBSTANTIATION FOR TITAN™ SPORTS SUPPLEMENT

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INFLAMMATION AND MUSCLE SORENESS

[001] The muscle tissues of animals, including humans, are in a constant state of flux between the anabolic processes that build up muscle tissues and the catabolic processes, which degrades muscles tissues. A state of health exists when there is a balance between these two processes and derangements of the balance produce disease. Skeletal muscle comprises approximately 40 per cent of the body while another 5 to 10 per cent is smooth and cardiac. Skeletal muscle tissue is of particular importance for several reasons. First, it allows mobility by the lengthening and contraction of muscle fibers in addition to providing support to joints. Second, it provides strength and allows work to be preformed. Third, it enhances the metabolic rate by approximately 50 calories per day for each pound of muscle gained.

[002] In order for muscles to maintain or gain strength, they must be exercised or used to a degree that is greater than what they are normally accustomed to. If one does the same exercises at the same pace every day, they will never become faster, stronger or have greater endurance. If one stops exercising when their muscles just start to burn from lactic acid production, they won't feel sore the next day and the muscles will not become stronger. All improvement in any muscle function comes from stressing and recovering – a process called over-reaching. Stressing the muscles consists of exercising hard enough that the muscles begin to burn, due to lactic acid buildup, and reach the point of exhaustion or muscle failure. On the next day, the muscles feel sore because they have become damaged with resulting inflammation and need time to recover, which usually takes 3 to 4 days. This post-exercise soreness is called delayed onset muscle soreness (“DOMS”) (Connolly, *et al.*, 2003; Cheung, *et al.*, 2003).

[003] It takes approximately eight hours, more or less, to feel this type of soreness. When one finishes a hard workout, their muscles will feel exhausted, but as the day passes they will start to feel sore and by the next day the DOMS can be anywhere from moderate to severe, depending on the intensity of the previous day's workout. DOMS is initiated by damage to the muscle fibers themselves. Muscle biopsies taken the day after intense exercise show bleeding and disruption of the z-band filaments that hold muscle fibers together as they slide over each other during a contraction (Cheung, *et al.*, 2003). This damage leads to acute inflammation, accumulation of metabolites (e.g. free radicals) that increase damage, fluid retention, minor connective tissue tears and a combination of all of the above.

[004] Following moderate to intense exercise, there is a disruption of the contractile component of the muscle tissue, particularly at the level of the z-line (Friden, *et al.*, 1981; Friden, *et al.*, 1983; Newham, *et al.*, 1983; Armstrong, 1984; Friden, *et*

al., 1984; Jones, *et al.*, 1986; Friden, *et al.*, 1988). The characteristic lesions that are observed microscopically are total myofibrillar disruption of the z-line in addition to more widespread disruption of sarcomere architecture (Newham, *et al.*, 1986; Friden and Lieber, 1992). This disruption to the structural components of the muscle is increased particularly amongst the type II muscle fibers, which have the narrowest and weakest z-lines. Creatine phosphokinase (“CPK”) is found exclusively in the muscles and leaks out into the bloodstream when muscles are damaged. CPK is considered a reliable indicator of muscle membrane damage, which leads to permeability. Therefore, those individuals who have the highest post-exercise blood levels of CPK will ultimately have the most muscle soreness.

[005] When z-lines are disrupted and the sarcolemma damaged, this allows the leakage of muscle enzymes, such as CPK, into the interstitial fluid that surrounds the cells. Following exercise, serum CPK levels have been shown to increase 400-times over normal levels (Newham, *et al.*, 1983) indicating disruption of the muscle cell membranes (Newham, *et al.*, 1986; Cleak, and Eston, 1992; Brown, *et al.*, 1997; Armstrong, 1990). Because there is a clear discrepancy between the observed times of peak CPK levels in the blood and muscle soreness (Newham, *et al.*, 1986; Walsh, *et al.*, 2001; Evans, *et al.*, 1986; Clarkson, and Ebbeling, 1988; Jones and Newham, 1985; Newham, *et al.*, 1983; Clarkson, *et al.*, 1986; Clarkson, *et al.*, 1986), muscle damage can only be viewed as a factor contributing to DOMS, but not the actual cause.

[006] The real cause of DOMS is due to the inflammatory response that brings about the presence of inflammatory cells and edema that are present at the site of injury following exercise (Francis and Hoobler, 1987; Evans, *et al.*, 1986; Stupka, *et al.*, 2000; Smith, 1991; Smith, 2001; MacIntyre, *et al.*, 2001). Muscles contain enzymes that can breakdown the membrane lipids and structural proteins following injury or damage. Following muscle tissue disruption by exercise, there is a release of intracellular calcium, which activates calcium-dependent proteolytic enzymes that degrade the z-lines, troponin and tropomyosin. This enzymatic breakdown of damaged muscle fibers is accompanied by a release and accumulation of bradykinin, histamine and prostaglandins, which are mediators of inflammation. These mediators help increase vascular permeability and attract neutrophils to the site of injury (Hasson, *et al.*, 1993). The leaking intracellular proteins serve as markers of muscle damage and attract monocytes, which turn into macrophages. Because blood vessels become more permeable due to the inflammatory mediators, protein-rich fluid (exudate) escapes from blood plasma and enters the damaged tissue. This protein rich fluid causes the muscle cells to take up water, resulting in edema, which gives rise to swelling and pain. Peak edema levels have been shown to coincide with peak DOMS (Lightfoot, *et al.*, 1997; Gulick and Kimura, 1996).

[007] Depending on an individual's level of fitness, use of the body's muscles beyond one's normal limits, such as repetitive squatting while working in the garden, running in a marathon, or a workout using progressive resistance weight training will result in DOMS. Depending on how sore muscles are the following day, work or athletic training is usually curtailed for one to four days or only done in moderation at the very least. For this reason, it is recommended that one not train until the muscles have

recovered and all muscle soreness has resolved. Therefore, marathon runners only run very hard and fast twice a week. Weightlifters only train the same muscle groups two times per week. The downside to DOMS is that it dictates a training schedule whereby two-thirds of the time is spent allowing muscles to recover. This amounts to a considerable amount of time where the athlete cannot train and is in a state of considerable discomfort.

[008] Gains in athletic performance, such as strength and endurance, can only be accomplished by working out more often and at greater intensity. If post-workout recovery time could be shortened by the reduction or elimination of DOMS, athletes would be able to spend more time training and less down time while recovering.

DIET, EXERCISE AND HORMONE INTERACTIONS

[009] When an individual begins pushing their muscles to the limit by exercising intensely for 45-60 minutes, the need for energy is enormous. During the first few minutes, the muscles burn up their existing energy stores of ATP, the molecule that provides the power for muscle contractions. The ATP can temporarily be replaced by creatine phosphate, but then muscle tissue starts to burn glucose and glycogen for fuel. As the workout continues, blood insulin levels begin to drop and the liver starts to release more glucose into the blood. The low levels of insulin also cause the fat cells to release fatty acids, which can be burned as fuel. Essentially, the body is mobilizing its fuel reserves to support the intense use of its muscles.

[010] Once the muscle activity ends, the body must begin to restore its depleted energy reserves and get blood glucose levels back to normal. After intense exercise, muscle glycogen stores can be completely depleted. To recover, one must take in nutrients, especially carbohydrates.

[011] Food is generally considered as a source of fuel, although nutrient ingestion following exercise results in hormonal responses that will interact with target tissues. Because the post-exercise diet can exert profound influence on specific hormones, which in turn can have an anabolic effect on target tissues, especially the muscles, both the timing and proper selection of nutrients can be very important. Optimizing the hormonal environment in favor of an anabolic profile during the recovery period between exercise sessions would be advantageous for promoting recovery and maximizing muscle adaptations to training (Volek and Forsythe, 2006).

[012] A prolonged state of intense exercise leaves the body in a hypoglycemic state, or with low levels of glucose. Following exercise, the glycogen stores in the muscle can be depleted. To replenish muscle glycogen, it is necessary to get carbohydrates back into the body as fast as possible, especially high glycemic carbohydrates (Walton and Rhodes, 1997; Haff, *et al.*, 2003; Burke, *et al.*, 2004; Ivy, 2004). During the first 30 minutes after exercise, the body starts the recovery phase by mobilizing all its resources for replacing glycogen and building new muscle proteins (Ivy, J.L., 1988). If the nutrients are not immediately available, then these processes turn off and recovery will take a much longer time.

[013] It has been shown that when a high glycemic carbohydrate supplement containing protein is given immediately after exercise, there is a rapid rise in the blood glucose levels, also known as hyperglycemia (Rabinowitz, *et al.*, 1966; Nuttall, *et al.*, 1984; Spiller, *et al.*, 1987; Zawadzki, *et al.*, 1992; Chandler, *et al.* 1994; Ivy, 2004). This carbohydrate-protein induced rise in blood insulin is greater than either carbohydrate or protein alone (van Loon, 2000). Within 30 minutes of taking the carbohydrate supplement, the pancreas starts to release large amounts of insulin to get these high levels of blood glucose under control. The insulin binds to receptors on the muscle cells and facilitates the rapid entry of glucose into the muscle cells for glycogen synthesis. These high plasma insulin levels will ultimately deplete the serum glucose to the extent that it will again deplete blood glucose levels and result in hypoglycemia.

[014] It would seem reasonable that when an individual rebounds into a state of hypoglycemia and insulin levels diminish, the body would enter into a catabolic state. However, it has been shown that the rapid rise and fall of blood glucose and insulin levels that are induced by a carbohydrate-protein supplement immediately after exercise causes a release of anabolic hormones after exercise, such as growth hormone (Roth, *et al.*, 1963; Kramer, *et al.*, 1990; Kraemer, *et al.*, 1991) and insulin-like growth factor (Kramer, *et al.*, 1990; Kraemer, *et al.*, 1991) that continues to promote protein synthesis. This is in addition to the testosterone that is released due to the lactate buildup in the blood from exercise (Fahey, *et al.*, 1976; Weiss, *et al.*, 1983; Kramer, *et al.*, 1990; Kraemer, *et al.*, 1991).

[015] During high-intensity exercise the concentrations of hormones in the blood and other body fluids can increase ten to twenty times over their levels at rest (Kraemer, 2000). At the same time that anabolic hormones are being released in response to exercise, catabolic hormones are also being released. Release of cortisol by the adrenal glands is directly linked to a variety of physiological functions, such as increased levels of proteolytic enzymes, inhibition of protein synthesis and the conversion of amino acids into carbohydrate. When muscle glycogen concentrations become low, blood cortisol levels increase to signal a need to shift to other energy substrates such as protein or fat so that judicious use is made of the little glucose that remains. While this is catabolic to muscles, the body is trying to preserve carbohydrate stores of glucose, which is the sole energy source for the brain. The body will literally cannibalize other tissues and organs to preserve glucose for the brain.

[016] This degradation and loss of muscle tissue would result in a loss of strength, speed and mobility, which from an evolutionary standpoint would have a negative impact on an individual's ability to survive in a hunter-gatherer society where procurement of food depended on one's ability to work and search for food. The release of cortisol as a physiological survival response is obviously a double-edged sword in that it preserves the brain at the expense of muscle. To counter muscle loss, the body developed an additional response. During exercise, anabolic hormones, such as testosterone, insulin, growth hormone and insulin-like growth factors are released to help maintain muscle as well as bone and connective tissues.

[017] Type I muscle fibers, or slow-twitch fibers, are generally fatigue resistant and have a high capacity for aerobic energy supply, but have limited potential for rapid force development. These fibers are best suited for aerobic work, such as running. Whereas endurance training does not provide a stimulus to increase the size of the Type I muscle fibers, these fibers do resist getting bigger with resistance training due to the need for optimal size for oxygen kinetics by down-regulating their testosterone receptors. At the same time, cortisol's influence is also diminished, both at the level of the receptor in the muscle and in the testes in men, which allows men to produce more testosterone. This reduced influence of cortisol to promote protein degradation and the influence of testosterone on protein synthesis results in hypertrophy of the Type I fibers in response to heavy resistance training. Therefore, type I muscle fibers gain more size by reducing the amount of protein degradation than by increasing the amount protein synthesis (Kraemer, 2000).

[018] Type II muscle fibers, or fast-twitch fibers, are fatigable, have low aerobic power and rapid force development. These fibers are best suited for anaerobic work, such as weight lifting. Type II muscle fibers increase in size more by increasing the amount of protein synthesis than by reducing degradation, although both take place. The testosterone receptors in these fibers are up regulated by resistance training and therefore can contribute to protein synthesis and an increase in fiber size, whereas aerobic training has no effect. Other hormones, such as insulin, growth hormone and insulin-growth factor-1 also participate in the growth of type II muscle fibers in response to exercise. About 50 per cent of muscle growth is thought to be due to growth hormone. Therefore, post-exercise nutritional supplementation that can support additional growth hormone release will greatly benefit gains in muscle mass and strength (Kraemer, 2000).

[019] Because muscles are in a dynamic state of equilibrium whereby work or exercise causes the release of both catabolic and anabolic hormones, the net gain or loss of muscle mass depends upon the ratio of catabolic to anabolic processes. In order to gain more muscle mass in response to weight training, athletes have tried various nutritional strategies aimed at increasing protein syntheses by increasing the cascade of anabolic hormones released and/or decreasing catabolism by preventing cortisol release.

[020] As alluded to above, it is known that consuming a carbohydrate-rich meal immediately after exercise will raise insulin levels and begin a metabolic cascade of anabolic hormones that will increase muscle growth. In one study four groups of experienced bodybuilders were given post-workout supplements that consisted of: (1) 3.3 g carbohydrate (dextrose and maltodextrin) per pound of body weight, (2) 3 g protein (whey) per pound of body weight, (3) 2.3 g carbohydrates and 0.9 g protein per pound of body weight, and (4) water was given as a control (Chandler, et al., 1994). The supplements were given immediately after and two hours after exercise. Insulin levels were increased at 30 minutes with the carbohydrate-protein supplement giving the greatest increase, followed by the carbohydrate supplement, then the protein supplement. The two-hour supplements had little effect on the insulin response and no effect on blood glucose levels, which reinforces the importance of supplementation

immediately following exercise. The resistance training caused a release of growth hormone, which declined to baseline levels within two hours. However, at five to six hours post-exercise the growth hormone levels spiked again and showed a significant rise in the carbohydrate-protein and carbohydrate supplemented individuals. This second surge of growth hormone returned to baseline with two hours. Testosterone also increased as a result of exercise, but declined to baseline levels by six hours in all subjects.

[021] The obvious benefit for these insulin surges is the anabolic effect it has on the muscle and its ability to increase protein synthesis and inhibit protein breakdown (Rooyackers and Nair, 1997). Insulin causes the rapid uptake of amino acids into the muscle to increase intracellular amino acid pools. It can also increase protein synthesis directly by increasing both transcription at the DNA level (Horovitz-Fried, *et al.*, 2006) and transcription at the RNA level (Wool and Cavicchi, 1966; Fahmy and Leader, 1978; O'Connor, *et al.*, 2003). Insulin can prevent protein degradation (Ashford and Pain, 1986; Balon, *et al.*, 1990) and this anti-catabolic effect is thought to be through regulation of lysosome activity (Long, *et al.*, 1984).

[022] Insulin is generally accepted to be a stimulator of protein synthesis only when adequate amino acids are available (Kimball, *et al.*, 2002), thus the need for supplemental essential amino acids following exercise. Because of this, much research has focused on the timing for post-workout supplementation to increase insulin release and examining which amino acids contribute most to protein synthesis following exercise.

[023] Infusion of amino acids into resting, fasting human subjects increases amino acid transport into the muscle cell, stimulates muscle protein synthesis and improves net nitrogen balance from negative to slightly positive values, but had no effect on muscle protein breakdown (Biolo, *et al.*, 1997). Resistance exercise also stimulates muscle protein synthesis; but muscle protein breakdown is also elevated so that, although net nitrogen balance increases, this balance remains negative (Biolo, 1995a). However, when an amino acid solution was infused into subjects after an hour of heavy resistance exercise, protein synthesis was increased to a greater extent than either exercise or the amino acid treatment alone (Biolo, 1997).

[024] The body uses twenty amino acids for protein synthesis and of these twenty, nine are essential for man (Whitney and Rolfes, 2002) as they cannot be made by the body and must be obtained in the diet or by other external means. In some cases, nonessential amino acids can become conditionally essential when circumstances arise whereby the body cannot produce the amounts required and these amino acids must be supplemented from an external source. The conditionally essential amino acids are arginine, cysteine, glycine and tyrosine. Ingestion of nonessential amino acids is not necessary for stimulation of muscle protein synthesis (Tipton, 1999) because the body can quickly make them and therefore it is only the essential amino acids, and sometimes the conditionally essential, that can limit muscle growth in response to exercise-induced protein synthesis.

[025] Based on the knowledge that the post-exercise infusion of amino acids has a positive effect on protein synthesis (Biolo, *et al.*, 1997) and that hyperinsulinemia stimulates amino acid uptake and protein synthesis (Biolo, *et al.*, 1995b), an oral drink which consisted of essential amino acids and high glycemic sugars (sucrose + maltodextrin) was tested for its effects on muscle protein synthesis. In a cross-over study, one group of healthy subjects were given either the essential amino acids with carbohydrate on one occasion followed by a placebo the next time, while the other group was given a placebo on one occasion followed by essential amino acids with carbohydrate on the next occasion. The supplements were given at one and three hours after resistance exercise. The essential amino acids/carbohydrate drink increased blood amino acids and insulin levels within 20 to 30 minutes when ingested at either one or three hours after resistance exercise. There was also an increase in net protein synthesis in the muscles within 20 minutes that returned to baseline within one hour. The placebo had no effect on insulin, amino acid levels or protein synthesis. Therefore, the essential amino acids/carbohydrate drink promoted anabolic conditions in the muscle beyond what could be achieved by exercise alone (Rasmussen, *et al.*, 2000).

[026] Although this increase in protein synthesis was transient, this was the highest protein synthetic rate observed under any circumstance and reflects a synergistic effect between the availability of essential amino acids, insulin and resistance training. The increase in muscle protein synthesis compared with resting values under various circumstances are as follows: (1) physiological hyperinsulinemia – 50% (Biolo, *et al.*, 1995b), (2) resistance exercise – 100% (Biolo, *et al.*, 1995b), (3) amino acid availability – 150% (Biolo, *et al.*, 1997b), (4) amino acid availability after resistance exercise – 200% (Biolo, *et al.*, 1997), (5) amino acid availability after resistance exercise with hyperinsulinemia – 400% (Rasmussen, *et al.*, 2000).

[027] The role of oral supplementation of amino acids in the prevention of DOMS is a field of interest for nutritional science that has a direct and practical application in the field of sports. Oral supplementation with branched chain amino acids (BCAA) before exercise has been reported to suppress endogenous muscle protein breakdown (MacLean, *et al.*, 1994). This finding was corroborated in another study whereby giving BCAA before, during after exercise showed a protein sparing effect on muscle proteins (Blomstrand and Saltin, 2001). Supplementation with BCAA for 2 weeks plus additional BCAA before and after exercise reduced serum CK for several days following exercise (Coombes *et al.*, 2000). A recent study investigated administering BCAA before exercise and reported a reduction on DOMS for several days following exercise (Shimomura, *et al.*, 2006). Previous studies have shown that administering EAA after exercise results in a change from net protein degradation to net protein synthesis (Tipton, *et al.*, 1999).

[028] Carbohydrate-amino acid mixtures have more recently been shown to help reduce muscle soreness. When a liquid carbohydrate beverage was consumed 10-minutes before and immediately after exercise it resulted in a more favorable anabolic response, although it did not alter the catabolic response (Thyfault, *et al.*, 2004). When a solution of carbohydrate + protein was consumed immediately following

exercise, there was a perceived reduction in soreness among athletes (Millard-Stafford, *et al.*, 2005). Administration of a carbohydrate solution containing EAA during weight resistance exercise suppressed cortisol release, stimulated insulin release and reduced myofibrillar protein degradation (Bird, *et al.*, 2006a, 2006b) and increased the anabolic response (Bird, *et al.*, 2006c).

[029] Mixtures of amino acids have also been reported to reduce DOMS. Mixes of amino acids have been shown to reduce serum CK activity following exercise (Ohtani, *et al.*, 2001; Kraemer, *et al.*, 2006). When an amino acid mixture containing predominately essential amino acids was given twice daily on the day of exercise and for 4 days following exercise, there was a statistically significant reduction in both serum CK levels and DOMS in the days following exercise (Nosakai, *et al.*, 2006).

[030] Since the early 1960's, leucine and its keto acid, alpha-ketoisocaproate ("KIC") has been the subject of research into the regulation and prevention of muscle protein breakdown (Nair, *et al.*, 2002; Frexes-Steed, *et al.*, 1992). Leucine is unique in that it is not only an essential amino acid, but it is also a branched chain amino acid, which is catabolized during exercise. However, in the early 1990s, a downstream metabolite called beta-hydroxy-beta-methylbutyrate ("HMB") was reported to have a positive effect on muscle protein balance (Nissen, *et al.*, 1996; Nissen, 2004).

[031] In the body, HMB is produced in the muscles and liver from the amino acid leucine (Sabourin and Bieber, 1981; Wagenmakers, *et al.*, 1985). It is also obtained in trace amounts from foods, with plants having the lowest concentrations and meats having the highest concentrations (Nissen, 2004). Although diet is a source of HMB, endogenous production of HMB from leucine generally far exceeds dietary intake. Therefore, foods containing large concentrations of leucine would probably have a greater influence on the circulating concentrations of HMB in the body. Studies where animals and humans have been given leucine intravenously have shown an increased rate of production and increased plasma levels of HMB (Van Koevering and Nissen, 1992).

[032] Early studies using a limited number of subjects appeared to indicate that HMB could alter body composition by partially preventing exercise-induced muscle fiber breakdown in athletes and therefore be anti-catabolic in nature (Nissen, *et al.*, 1994). However, subsequent more-well-controlled studies have clearly shown that HMB has no effect on preventing muscle catabolism, altering body composition or increasing strength in athletes (Kreider, *et al.*, 1999; Kreider, *et al.*, 2000; Gallagher, *et al.*, 2000; Paddon-Jones, *et al.*, 2001; Slater, *et al.*, 2001; Ransone, *et al.*, 2003; Thomson, 2004) and clearly has not lived up to it's early anti-catabolic claim.

[033] A number of studies have investigated the effects of HMB on muscle damage following a single bout of strenuous exercise (Knitter, *et al.*, 2000). HMB supplementation has been shown to reduce the appearance of CPK and lactate dehydrogenase ("LDH"), both indicators of muscle damage (Nissen, 2004). Both CPK and LDH are muscle enzymes that appear in the blood following muscle membrane damage and the amount in the blood is proportional to the severity of the damage.

When runners were given either HMB or a placebo during six weeks of training followed by a 20 km run, blood samples taken after the run showed that the HMB-supplemented individuals had reduced levels of CPK and LDH compared to the placebo control subjects (Knitter, *et al.*, 2000). Using a downhill running protocol, it was shown that supplementation with HMB reduced the amount of perceived muscle soreness and resulting in less strength loss. This suggests an anti-inflammatory effect on muscles, which results in less soreness (Byrd, *et al.*, 1999).

[034] Other studies have investigated the effects of HMB on preventing muscle membrane damage following an intense resistance-training program (Nissen *et al.*, 1996; Kreider, *et al.*, 1999; Panton, *et al.*, 2000; Gallagher, *et al.*, 2000; Jówko, *et al.*, 2001). Men and women who took part in a 4-week weight training program and supplemented with HMB showed a 2% decrease in blood CPK levels while those using a placebo showed a 26% increase in CPK levels due to the weight training (Panton, *et al.*, 2000). 3-methylhistidine (“3-MH”) is also a marker of muscle damage and when subjects undergoing intense resistance-weight training were supplemented with HMB there was a significant decrease in plasma 3-MH (Nissen *et al.*, 1996). These findings suggest HMB supplementation minimizes the leakage of soluble proteins that occurs after intense exercise.

[035] In the body, HMB is metabolized into beta-hydroxy-beta-methylglutaryl-CoA (HMG-CoA), which is used for the production of cholesterol (Nissen and Abumrad, 1997). In muscle cells, *de novo* cholesterol synthesis appears to be the major if not exclusive sources of cholesterol (Nissen and Abumrad, 1997). During exercise when extensive muscle membrane damage is occurring, intracellular levels of cholesterol, which is necessary for membrane repair, may be limited. Because HMB can lead to increased muscle cholesterol synthesis, this may allow muscle cells to rapidly repair their membrane damage following exercise and prevents release of soluble proteins that leads to inflammation. A recent study in patients with chronic obstructive pulmonary disease (COPA) have documented that HMB has an anti-inflammatory effect as evidenced by reduced C-reactive protein and improved pulmonary function (Hsieh, *et al.*, 2006).

[036] In one study, plasma HMB levels were measured when HMB was administered with water or with 75 g glucose in water (Vukovich, *et al.*, 2001). The results showed that both groups had identical blood glucose and insulin levels, but the group who consumed HMB with glucose had lower plasma HMB levels and less HMB excreted in the urine. Their conclusion, although erroneous, was that the glucose slowed gastric emptying and prevented HMB from entering the blood as fast.

[037] The studies by Vukovich, *et al.* (2001) failed to prove that when HMB is taken with high glycemic sugars, such as glucose, a reduction in absorption of HMB is responsible for lower plasma HMB levels. In contrast, the Titan™ Sports Supplement which is composed of high-glycemic sugars and/or carbohydrates, essential amino acids and HMB rapidly enters the muscles whereby it prevents or greatly reduces DOMS when taken immediately following exercise. A contributing factor is most likely that because HMB is a leucine amino acid metabolite, the insulin response caused by

the sugar helps rapidly drive the HMB into the muscles. In Vukovich's study, it is more likely that plasma HMB levels were lower in the presence of glucose because the elevated insulin levels facilitated the entry of HMB into the muscle thus clearing it more rapidly from the blood. This accelerated entry of HMB into the muscle, results in addition cholesterol reserves to initiate faster repair of damaged membranes. This results in a greater reduction in membrane permeability, less leakage of soluble muscle proteins and the resulting inflammation, than when HMB is taken alone. The essential amino acids, which have also been shown to elicit an anabolic response and contribute to a reduction in soreness when taken with carbohydrate, act in conjunction with the HMB to provide a synergistic response that results in the prevention or significant reduction in DOMS.

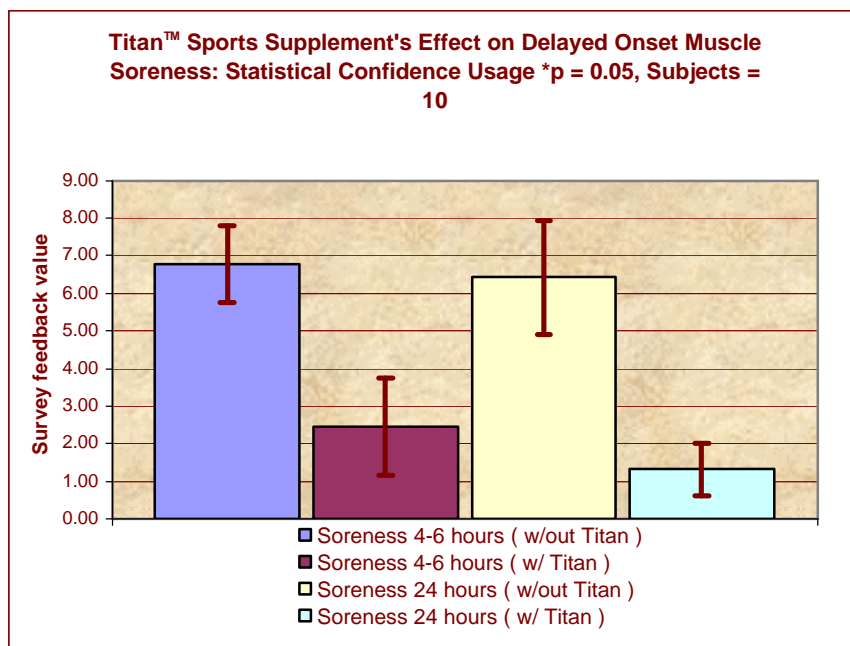
[038] A recent study has described the use of HMB in the prevention of DOMS (van Someren, *et al.*, 2005). In this study, eight males who had not exercised in a year were administered HMB/KIC for 14 days prior to a single bout of exercise. After completing a muscle damaging exercise protocol of eccentric resistance training, it was determined that CPK and DOMS were significantly reduced after 14 days supplementation with HMB/KIC compared to controls. One previous study that looked at the effects of short term HMB supplementation on eccentric resistance training found that six days supplementation with HMB did not reduce DOMS after a single bout of exercise. Therefore, it would appear that prevention of DOMS by supplementation with HMB alone requires supplementation for more than 6 days prior to exercise. This supports a role for HMB in the prevention of muscle soreness, but also demonstrates how the Titan™ proprietary blend, which consists of CHO/EAA/HMB, is more effective at immediately reducing DOMS than HMB alone.

[039] Caffeine has also been shown to help alleviate muscle pain during exercise (Morl, *et al.*, 2003; O'Connor, *et al.*, 2004; Motl, *et al.*, 2006) as well as DOMS (Maridakis, *et al.*, 2007) at moderate doses of 350 to 700 mg, although the effect was not dose dependent. It has also been shown in moderate amounts to help increase intestinal absorption of glucose (van Nieuwenhoven, *et al.*, 2000; Yeo, *et al.*, 2005) and increase the insulin response over that of glucose alone (Graham, *et al.*, 2001). It has also been reported that exercise helps alleviate the anxiety that some people experience with caffeine use (Youngstedt, *et al.*, 1998).

TITAN™ SPORTS SUPPLEMENT STUDY

[040] In a preliminary investigation (Sparkman, unpublished results), eight normal healthy individuals of both sexes with an age range of 24 - 55 years took part in various types of aerobic or anaerobic exercise (weight training, running, kickboxing) to such a degree as to insure that DOMS would ensue the following day. After the first training session, each individual immediately drank 500 ml of Titan™ Sports Supplement. A questionnaire was filled out over the following 24 hours post exercise. The following week, the same individuals took part in the same exercise program they had preformed the previously week for the same time and using the same intensity in their workout, but without using Titan™ Sports Supplement. Again, a questionnaire was completed over the following 24 hours post exercise. The questionnaire asked the

individuals to rate how they felt both physically and mentally using a visual analogue scale (VAS). All individuals reported extreme muscle exhaustion and feeling extremely tired after both workouts. After the first workout using Titan™ Sports Supplement, all individuals reported little to no muscle soreness at approximately six and 24 hours post-exercise and most felt very good. None of the individuals used any other supplements or analgesics during the study. After the second workout where the individuals did not use Titan™ Sports Supplement, all individuals reported muscle soreness at approximately six and 24 hours post-exercise and mood varied from good to bad. One individual had to use analgesics to help deal with the soreness. All individuals reported that they experienced less delayed onset muscle soreness and felt better following their workout using Titan™ Sports Supplement.



GASTRIC EMPTYING AND OSMOLARITY

[041] The rate at which the stomach empties is regulated by signals from both the stomach and duodenum, including nervous signals caused by distention of the stomach and release of the hormone gastrin from the antral mucosa. Both these signals increase the pyloric pumping force and inhibit the pylorus from closing. Although the pylorus normally remains closed, the closing force is weak enough to allow water and other fluids to empty from the stomach. When the carbohydrate concentration in a beverage is 8% or higher, the rate of gastric emptying slows (Murray, *et al.* 1999; Shi, *et al.* 2004). The carbohydrate concentration of this sports supplement is 7% carbohydrate to promote rapid gastric emptying.

[042] Gastric emptying is greatly influenced by its volume. The rate of gastric emptying decreases exponentially as fluid volume is depleted. Therefore, an effective way to speed gastric emptying is by maintaining high fluid volumes in the stomach. In humans, the stomach has a volume of about 50 mL when empty. After a meal, it

generally expands to hold about 1 liter of food, but it can actually expand to hold as much as 4 L. The volume of this sports supplement is 500 ml, which is sufficient to expand the stomach enough to promote gastric emptying.

[043] Another factor that promotes gastric emptying is rate of digestion. A prompt digestion rate means that nutrients can transport swiftly and efficiently from the stomach to the intestines where the digested nutrients are absorbed into the bloodstream and transported to the muscles. It has been shown that this process slows when the ingested fluid contains a high osmolarity. Osmolarity is dependent on the number of particles in a solution. Thus, a solution with a high concentration of glucose will have a higher osmolarity than a solution that only contains both glucose and complex polysaccharides. The shorter chain length a carbohydrate has, the higher it raises the solution's osmolarity. Therefore, it is no surprise that a pure glucose solution (monosaccharides) induces very high concentrations of solute (Beckers, *et al*, 1992; Duchman, *et al.*, 1997; Schedl, *et al.*, 1994). Providing that the osmolarity is near isotonic, solutions pass quickly out of the stomach and into the small intestines. Here disaccharides, such as sucrose, are digested by intestinal epithelial cell sucrase into the monosaccharides glucose and fructose. These monosaccharides are then immediately absorbed into the portal blood. Titan™ Sports Supplement employs maltodextrin to bring the osmolarity to near isotonicity to promote rapid gastric emptying, whereby the sucrose is digested and glucose rapidly absorbed into the blood. The amino acids and HMB in this Sports Supplement are free form and require no further digestion. Therefore, after being rapidly emptied into the small intestine, they are immediately absorbed into the blood.

[044] Studies have shown that when HMB and amino acids are co-administered, they are rapidly absorbed into the bloodstream within 20 to 30 minutes (Rasmussen, *et al.*, 2000; Vikovich, *et al.*, 2001).

REFERENCES

Armstrong, R.B. Mechanisms of exercise-induced delayed onset muscle soreness: a brief review. *Med. Sci. Sports Med.* 16: 529-538, 1984.

Armstrong, R.B. Initial events in exercise-induced muscular injury. *Med. Sci. Sports Exerc.* 22: 429-435, 1990.

Ashford, A.J., Pain, V.M. Insulin stimulation of growth in diabetic rats. Synthesis and degradation of ribosomes and total tissue protein in skeletal muscle and heart. *J. Biol. Chem.* 25: 4066-4070, 1986.

Balon, T.W., Zorzano, A., Treadway, J.L., Goodman, M.N., Ruderman, N.B. Effect of insulin on protein synthesis and degradation in skeletal muscle after exercise. *Am. J. Physiol.* 258: E92-E97, 1990.

Beckers, E.J., Leiper, J.B., Davidson, J. Comparison of aspiration and scintigraphic techniques for the measurement of gastric emptying rates of liquids in humans. *Gut* 33:115-117,1992.

Biolo, G., Williams, B.D., Tipton, K.D., Wolfe, R.R. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am. J. Physiol. Endocrinol. Metab.* 268: E514-520, 1995a.

Biolo, G., Declan Fleming, R.Y., Wolfe, R.R. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of amino acids into human skeletal muscle. *J. Clin. Invest.* 95: 811-819, 1995b.

Biolo, G., Tipton, K.D., Klein, S., Wolfe, R.R. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am. J. Physiol. Endocrinol. Metab.* 273: E122-E129, 1997.

Bird, S.P., Tarpinning, K.M., Marino, F.E. Liquid carbohydrate/essential amino acid ingestion during a short-term bout of resistance exercise suppresses myofibrillar protein degradation. *Metabolism* 55: 570-577, 2006a.

Bird, S.P., Tarpinning, K.M., Marino, F.E. Independent and combined effects of liquid carbohydrate/essential amino acid ingestion on hormonal and muscular adaptations following resistance training in untrained men. *Eur. J. Appl. Physiol.* 97: 225-238, 2006c.

Bird, S.P., Tarpinning, K.M., Marino, F.E. Effects of liquid carbohydrate/essential amino acid ingestion on acute hormonal response during a single bout of resistance exercise in untrained men. *Nutrition* 22: 367-375, 2006b.

Byrd, P.L., Mehta, P.M., DeVita, P., Dych, D., Hickner, R.C. Changes in muscle soreness following downhill running: effects of creatine, HMB, and betagen supplementation. *Med. Sci. Sports Exer.* 31: S263, 1999.

Blomstrand, E., Saltin, B. BCAA intake affects protein metabolism in muscle after but not during exercise in humans. *Am. J. Physiol. Endocrinol. Metab.* 281: E365-E374, 2001.

Brown, S.J., Child, R.B., Day, S.H. Indices of skeletal muscle damage and connective tissue breakdown following eccentric muscle contractions. *Eur. J. Appl. Occup. Physiol.* 75: 369-374, 1997.

Burke, L.M., Kiens, B., Ivy, J.L. Carbohydrates and fat for training and recovery. *J. Sports Sci.* 22: 5-30, 2004.

Chandler, R.M., Byrne, H.K., Patterson, J.G., Ivy, J.L. Dietary supplements affect the anabolic hormones after weight training exercise. *J. Appl. Physiol.* 76: 839-845, 1994.

Cheung, K., Hume, P.A., Maxwell, L. Delayed onset muscle soreness. Treatment strategies and performance factors. *Sports Med.* 33: 145-164, 2003.

Clarkson, P.M., Apple, F.S., Byrnes, W.C., *et al.* Creatine kinase isoforms following isometric exercise. *Muscle Nerve* 10: 41-44, 1986.

Clarkson, P.M., Byrnes, W.C., McCormick, K.M., *et al.* Muscle soreness and serum creatine kinase activity following isometric, eccentric and concentric exercise. *Int. J. Sports med.* 7: 152-155, 1986.

Clarkson, P.M., Ebbeling, C. Investigation of serum creatine kinase variability after muscle damaging exercise. *Clin. Sci.* 75: 257-261, 1988.

Cleak, M.J., Easton, R.G. Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise. *Br. J. Sports Med.* 26: 267-272, 1992.

Connolly, D.A.J., Sayers, S.P., McHugh, M.P. Treatment and prevention of delayed onset muscle soreness. *J. Strength Cond. Res.* 17: 197-208, 2003.

Coombs, J.S., McNaughton, L.R. Effects of branched-chain amino acid supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise. *J. Sports Med. Phys. Fitness* 40: 240-246, 2000.

Duchman, S.M., Ryan, A.J., Schedl, H.P., Summers, R.W., Bleiler, T.L., Gisolfi, C.V. Upper limit for intestinal absorption of a dilute glucose solution in men at rest. *Med. Sci. Sports Exercise* 29: 482-488, 1997.

Evans, W.J., Meredit, C.N., Cannon, J.G., *et al.* Metabolic changes following eccentric exercise in trained and untrained men. *J. Appl. Physiol.* 61: 1864-1868, 1986.

Fahey, T., Rolph, R., Moungmee, P., Nigel, J., Mortara, S. Serum testosterone body composition and strength of young adults. *Med. Sci. Sports* 8: 31-34, 1976.

Fahmy, L.H., Leader, D.P. Insulin regulates the translation of rat skeletal-muscle messenger ribonucleic acid. *Biochem. Soc. Trans.* 6: 751, 1978.

Francis, K.T., Hoobler, T. Effects of aspirin on delayed onset muscle soreness. *J. Sports med. Phy. Fitness* 27: 333-337, 1987.

Frexes-Steed, M., Lacy, D.B., Collins, J., Abumrad, N.N. Role of leucine and other amino acids in regulating protein metabolism *in vivo*. *Am. J. Physiol. (Endocrinol. Metabol.)* 262: E925-E935, 1992.

Friden, J., Kjorell, U., Thornell, L.E. Delayed onset muscle soreness and cytoskeletal alterations: an immunocytological study in man. *Int. J. Sports Med.* 5: 15-18, 1984.

Friden, J., Lieber, R.L. Structural and mechanical basis of exercise induced muscle injury. *Med. Sci. Sports Exerc.* 24: 521-530, 1992.

Friden, J., Seger, J., Ekblom, B. Sublethal muscle fiber injuries after high-tension anaerobic exercise. *Eur. J. Appl. Physiol.* 57: 360-368, 1988

Friden, J., Sjostrom, M., Ekblom, B. A morphological study of delayed onset muscle soreness. *Experientia* 37: 506-507, 1981.

Friden, J., Sjostrom, M., Ekblom, B. Myofibrillar damage following intense eccentric exercise in man. *Int. J. Sports Med.* 4: 170-176, 1983.

Gallagher, P.M., Carrithers, J.A., Godard, M.P., Schulze, K.E., Trappe, S.W. β -hydroxy- β -methylbutyrate ingestion, Part I: Effects on strength and fat free mass. *Med. Sci. Sports Exerc.* 32: 2109-2115, 2000.

Graham, T.E., Sathasivam, P., Rowland, M., Marko, N., Greer, F., Battram, D. Caffeine ingestion elevates plasma insulin response in humans during an oral glucose tolerance test. *Can. J. Physiol Pharmacol.* 79: 559-565, 2001.

Gulick, D.T., Kimura, I.F. Delayed onset muscle soreness: what is it and how do we treat it? *J. Sports Rehab* 5: 234-243, 1996.

Guyton, A.C. Insulin, glucagon and diabetes mellitus. In: *Textbook of Medical Physiology*, 6th Ed. Philadelphia, PA: W.B. Sanders; 1981: 959-972.

Haff, G.G., Lehmkuhl, M.J., McCoy, L.B., Stone, M.H. Carbohydrate supplementation and resistance training. *J. Strength Cond. Res.* 17: 187-196, 2003.

Hanson, S.M., Daniels, J.C., Divine, J.G., *et al.* Effect of ibuprofen use on muscle soreness, damage, and performance: a preliminary investigation. *Med. Sci. Sports Exerc.* 25: 9-17, 1993.

Horovitz-Fried, M., Jacob, A.I., Cooper, D.R., Sampson, S.R. Activation of the nuclear transcription factor SP-1 by insulin rapidly increases the expression of protein kinase C delta in skeletal muscle. *Cell Signal.* 19: 556-562, 2007.

Hsieh, L.C., Chien, S.L., Huang, M.S., Tseng, H.F., Chang, C.K. Anti-inflammatory and anti-catabolic effect of short-term beta-hydroxy-beta-methylbutyrate supplementation on chronic pulmonary disease patients in intensive care unit. *Asia Pac. J. Clin. Nutr.* 15: 544-550, 2006.

Ivy, J.L. Regulation of muscle glycogen repletion, muscle protein synthesis and repair following exercise. *J. Sports Sci. Med.* 3: 131-138, 2004.

Ivy, J.L., Katz, A.L., Cutler, C.L., Sherman, W.M., Coyle, E.F. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. *J. Appl. Physiol.* 93: 1480-1485, 1988.

Jones, D.A., Newham, D.J. The effect of training on human muscle pain and damage. *J. Physiol.* 365: 76, 1985.

Jones, D.A., Newham, D.J., Round, J.M., *et al.* Experimental human muscle damage: morphological changes in relation to other indices of damage. *J. Physiol.* 375: 435-448, 1986.

Jówko, E., Ostaszewski, P., Jank, M., Sacharuk, J., Zieniewicz, A., Wilczak, J., Nissen, S. Creatine and β -hydroxy- β -methylbutyrate (HMB) additively increases lean body mass and muscle strength during a weight training program. *Nutrition* 17: 558-566, 2001.

Kimball, S.R., Farrell, P.A., Jefferson, L.S. Role of insulin in transnational control of protein synthesis in skeletal muscle by amino acids or exercise. *J. Appl. Physiol.* 93: 1168-1180, 2002.

Knitter, A.E., Panton, L., Rathmacher, J. A., Petersen, A., Sharp, R. Effects of β -hydroxy- β -methylbutyrate on muscle damage following a prolonged run. *J. Applied Physiol.* 89: 1340-1344, 2000.

Kraemer, W.J., Physiological adaptations to anaerobic and aerobic endurance training programs. In: *Essentials of Strength Training and Conditioning*, 2nd ed. T.R. Baechle and R.W. Earle, Eds. Human Kinetics, Champaign, IL. 2000: 137-168.

Kraemer, W.J., Gordon, S.E., Fleck, S.J., Marchitelli LJ, Mello R, Dziados JE, Friedl K, Harman E, Maresh C, Fry AC. Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *Intl. J. Sports Med.* 12: 288-235, 1991.

Kraemer, W.J., Marchitelli, J.L., Gordon, S.E., Harman, E., Dziados, J.E., Mello, R., Frykman, P., McCurry, D., Fleck, S.J. Hormonal and growth factor response to heavy resistance exercise protocols. *J. Appl. Physiol.* 69: 1442-1450, 1990.

Kraemer, W.J., Ratamess, J.S., Volek, J.S., *et al.* The effect of amino acid supplementation on hormonal responses to resistance training overreaching. *Metabolism* 55: 282-291, 2006.

Kreider, R.B., Ferreira, M., Greenwood, M., *et al.* Effects of calcium β -HMB supplementation during training on markers of catabolism, body composition, strength and sprint performance. *J. Exerc. Physiol.* 3: 48-59, 2000.

Kreider, R.B., Ferreira, M., Wilson, M., Almada, A.L. Effects of calcium beta-hydroxy-beta-methylbutyrate (HMB) supplementation during resistance –training on markers of catabolism, body composition and strength. *Intl. J. Sports Med.* 20: 503-509, 1999.

Lightfoot, J.T., Char, D., McDermott, J., *et al.* Immediate post-exercise massage does not attenuate delayed onset muscle soreness. *J. Strength Cond. Res.* 11: 119-124, 1997.

Long, W.M., Chua, B.H., Munger, B.L., Morgan, H.E. Effects of insulin on cardiac lysosomes and protein degradation. *Fed. Proc.* 43: 1295-1300, 1984.

MacIntyre, D.L., Sorichter, S., Mair, J., Berg, A., McKenzie, D.C. Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *Eur. J. Appl. Physiol.* 84: 180-186, 2001.

Maridakis, V., O'Connor, P.J., Dudley, G.A., McCully, K.K. Caffeine attenuates delayed-onset muscle pain and force loss following eccentric exercise. *J. Pain* 8: 237-243, 2007.

McLean, D.A., Graham, T.E., Saltin, B. Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. *Am. J. Physiol. Endocrinol. Metab.* 267: E1010-E1022, 1994.

Millard-Stafford, M., Warren, G.L., Thomas, L.M., Doyle, J.A., Snow, T., Hitchcock, K. Recovery from run training: efficacy of a carbohydrate-protein beverage. *Int. J. Sport Nutr. Exerc. Metab.* 15: 610-624, 2005.

Motl, R.W., O'Connor, P.J., Dishman, R.K. Effect of caffeine on perceptions of leg muscle pain during moderate intensity cycling exercise. *J. Pain* 4: 316-321, 2003.

- Motl, R.W., O'Connor, P.J., Tubandt, L., Puetz, T., Ely, M.R. Effect of caffeine on leg muscle pain during cycling exercise among females. *Med. Sci, Sports Exerc.* 38: 598-604, 2006.
- Murray, R., Bartoli, W., Stofan, J., Horn, M., Eddy, D. A comparison of the gastric emptying characteristics of selected sports drinks. *Int. J. Sports Nutr.* 9: 263-274, 1999.
- Nair, K.S., Schwartz, R.G., Welle, S. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am. J. Physiol.* 263: E928-E934, 1992.
- Newham, D.J., Jones, D.A., Edwards, R.H.T. Plasma creatine kinase changes after eccentric and concentric contractions. *Muscle Nerve* 9: 59-63, 1986.
- Newham, D.J., Mills, K.R., Edwards, R.H.T. Large delayed plasma creatine kinase changes after stepping exercise. *Muscle Nerve* 6: 380-385, 1983.
- Nissen, S., Abumrad, N.N. Nutritional role of the leucine metabolite β -hydroxy- β -methylbutyrate (HMB). *J. Nutr. Biochem.* 8: 300-311, 1997.
- Nissen, S., Sharp, R., Ray, M., Rathmacher, J.A., Rice, D., Fuller, J.C. Jr, Connelly, A.S., Abumrad, N. The effect of the leucine metabolite β -hydroxy- β -methylbutyrate on muscle metabolism during resistance-exercise training. *J. Appl. Physiol.* 81: 2095-2104, 1996.
- Nissen, S. β -hydroxy- β -methylbutyrate. In: Wolinsky, I & Driskell, J.A., Eds. *Nutritional Ergogenic Aids.* Boca Raton, Florida: CRC Press; 2004: 147-170.
- Nosaka, K., Sacco, P., Mawatari, K. Effects of amino acid supplementation on muscle soreness and damage. *Int. J. Sport Nutr. Exer. Metab.* 16: 620-635, 2006.
- Nuttall, F.Q., Mooradian, A.D., Gannon, M.C., Billington C, Krezowski P. Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. *Diabetes Care* 7: 465-470, 1984.
- O'Connor, P.J., Motl, R.W., Broglio, S.P., Ely, M.R. Dose-dependent effect of caffeine on reducing leg muscle pain during cycling exercise is unrelated to systolic blood pressure. *Pain* 109: 291-298, 2004.
- O'Connor, P.M., Kimball, S.R., Suryawan, A., Bush, J.A., Nguyen, H.V., Jefferson, L.S., Davis, T.A. Regulation of translation initiation by insulin and amino acids in skeletal muscle of neonatal pigs. *Am. J. Physiol. Endocrinol. Metab.* 285: E40-E53, 2003.
- Ohtani, M., Maruyama K., Suzuki, S., Sugita, M., Kobayshi, K. Changes in hematological parameters of athletes after receiving daily dose of a mixture of 12 amino acids for one month during the middle- and long-distance running training. *Biosci. Biotechnol. Biochem.* 65: 348-355, 2001.

Paddon-Jones, D., Keech, A., Jenkins, D. Short-term beta-hydroxy-beta-methylbutyrate supplementation does not reduce symptoms of eccentric muscle damage. *Int. J. Sport Nutr. Exerc. Metab.* 11: 442-450, 2001.

Panton, L.B., Rathmacher, J.A., Baier, S., Nissen, S. Nutritional supplementation of the leucine metabolite β -hydroxy- β -methylbutyrate (HMB) during resistance training. *Nutrition* 16: 734-739, 2000.

Rabinowitz, D., Merimee, T.J., Maffezzoli, R., Burgess, J.A. Patterns of hormone release after glucose, protein, and glucose plus protein. *Lancet* 2: 454-457, 1966.

Ransone, J., Neighbors, K., Lefavi, R., Chromiak, J. The effect of beta-hydroxy-beta-methylbutyrate on muscular strength and body composition in collegiate football players. *J. Strength Cond. Res.* 17: 34-39, 2003.

Rasmussen, B.B, Tipton, K.D., Miller, S.L., Wolf, S.E., Wolfe, R.R. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J. Appl. Physiol.* 88: 386-392, 2000.

Rooyackers, O.E., Nair, K.S. Hormonal regulation of human muscle protein metabolism. *Ann. Rev. Nutr.* 17: 457-485, 1997.

Roth, J., Glick, S.M., Yalow, R.S., Berson, S.A. Hypoglycaemia: a potent stimulus of growth hormone. *Science* 140: 987-988, 1963.

Sabourin, P.J. and Bieber, L.L. Formation of β -hydroxyisovalerate from α -ketoisocaproate by a soluble preparation from rat liver. *Dev. Biochem.* 18: 149-154, 1981.

Schedl HP, Maughan RJ, Gisolfi CV. Intestinal absorption during rest and exercise: implications for formulating an oral re-hydration solution (ORS). *Med. Sci. Sports Exerc.*, 26: 267-280, 1994.

Shi, X., Horn, M.K., Osterberg, K.L., Stofan, J.R., Zachwieja, J.J., Horswill, C.A., Passe, D.H., Murray, R. Gastrointestinal discomfort during intermittent high-intensity exercise: effect of carbohydrate-electrolyte beverage. *Int. J. Sport Nutr. Exerc. Metab.* 14: 673-683, 2004.

Shimomura, Y., Yamamoto, Y., Bajotto, G., *et al.* Nutraceutical effects of branched-chain amino acids on skeletal muscle. *J. Nutr.* 136: 529S-532S, 2006.

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 athletes. *Int. J. Sports Nutr. Exerc. Metab.* 11: 384-396, 2001.

Smith, L.L., Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med. Sci. Sports Exer.* 23: 542-551, 1991.

Spiller, G.A., Jensen, C.D., Pattison, T.S., et al. Effect of protein dose on serum glucose and insulin response to sugars. *Am. J. Clin. Nutr.* 46: 474-480, 1987.

Stupka, N., Lowther, S., Chorneyko, K., Bourgeois, J.M., Hogben, C., Tarnopolsky, M.A. Gender differences in muscle inflammation after eccentric exercise. *J. Appl. Physiol.* 89: 2325-2332, 2000.

Thomson, J.S. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation of resistance trained men. *Asia Pac. J. Clin. Nutr.* 13: S59, 2004.

Thyfault, J.P., Carper, M.J., Richmond, S.R., Hulver, M.W., Pottleiger, J.A. Effects of liquid carbohydrate ingestion on markers of anabolism following high-intensity resistance exercise. *J. Strength Cond. Res.* 18: 174-179, 2004.

Tipton, K.D., Ferrando, A.A., Phillips, S.M., Doyle Jr., D, Wolfe, R.R. Postexercise net protein synthesis in human muscle from orally administered amino acids.

Tipton, K.D., Gurkin, B.E., Matin, S., Wolfe, R.R. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *J. Nutr. Biochem.* 10: 89-95, 1999.

Vanhelder, W.P., Radomski, M.W., Goode, R.C. Growth hormone response during intermittent weightlifting exercise in men. *Eur. J. Appl. Physiol. Occup. Physiol.* 53: 31-43, 1984.

Van Koevering, M., Nissen, S. Oxidation of leucine and alpha-ketoisocaproate to beta-hydroxy-beta-methylbutyrate *in vivo*. *Am. J. Physiol.* 262: E27-E31, 1992.

Van Loon, L.J., Saris, W.H., Kruijshoop, M., Wagenmakers, A.J. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *J. Clin. Nutr.* 72: 106-111, 2000.

Van Nieuwenhoven, M.A., Brummer, R.M., Brouns, F. Gastrointestinal function during exercise: comparison of water, sports drink, and sports drink with caffeine. *J. Appl. Physiol.* 89: 1079-1085, 2000.

Van Someren, K.A., Edwards, A.J., Howatson, G. Supplementation with β -hydroxy- β -methylbutyrate (HMB) and α -ketoisocaproic acid (KIC) reduces signs and symptoms of exercise-induced muscle damage in man. *Int. J. Sport Nutr. Exer. Metab.* 15: 413-424, 2005.

Volek, J.S., Forsythe, C.E. Diet, exercise and hormonal interactions on skeletal muscle. *Horm. Res.* 66: 17-21, 2006.

Vukovich, M.D., Slater, G., Macchi, M.B., Turner, M.J., Fallon, K., Boston, T., Rathmacher, J. β -hydroxy- β -methylbutyrate (HMB) kinetics and the influence of glucose ingestion in humans. *J. Nutr. Biochem.* 12: 631-639, 2001.

Wagenmakers, A.J., Salden, H.J., Veerkamp, J.H. The metabolic fate of branched chain amino acids and 2-oxo acids in rat muscle homogenates and diaphragms. *Int. J. Biochem.* 17: 957-965, 1985.

Walsh, B., Tonkonogi, M., Nalm, C., *et al.* Effect of eccentric exercise on muscle oxidative metabolism in humans. *Med. Sci. Sports Exerc.* 33: 436-441, 2001.

Walton, P., Rhodes, E.C. Glycaemic index and optimum performance. *Sports Med.* 23: 164-172, 1997.

Weiss, L.W., Cureton, K.J., Thompson, F.N. Comparison of serum testosterone and androstenedion response to weightlifting in men and women. *Eur. J. Appl. Physiol. Occup. Physiol.* 50: 413-419, 1983.

Whitney, E.N & Rolfes, S. R., In *Understanding Nutrition*, 9th ed., Wadworth, 2002, pp 167-195.

Wool, I.G., Cavicchi, P. Insulin regulation of protein synthesis by muscle ribosomes: Effect of the hormone on translation of messenger RNA for a regulatory protein. *Proc. Natl. Acad. Sci. USA* 56: 991-998, 1966.

Yeo, S.E., Jentjens, R.L.P.G., Wallis, G.A., Jeukendrup, A.E. Caffeine increases exogenous carbohydrate oxidation during exercise. *J. Appl. Physiol.* 99: 844-850, 2005.

Youngstedt, S.D., O'Connor, P.J., Crabbe, J.B., Dishman, R.K. Acute exercise reduces caffeine-induced angiogenesis. *Med. Sci. Sports Exerc.* 30: 740-745, 1998.

Zawadzki, K.M., Yaspelkis, B.B., Ivy, J.L. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. *J. Appl. Physiol.* 72: 1854-1859, 1992.