Effect of Carbohydrate-Protein Beverage on Glycogen Resynthesis and Muscle Damage Induced By Eccentric Resistance Exercise

Janet Regina Wojcik

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Janet Walberg Rankin, Ph.D., Chair
F. C. Gwazdauskas, Ph.D.
Robert S. Schulman, Ph.D.
Forrest Thye, Ph.D.
Jay H. Williams, Ph.D.

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(Abstract)

This study examined effects of carbohydrate (C), carbohydrate-protein (CP), or placebo (P) beverages following eccentric resistance exercise on muscle damage by serum creatine kinase (CK), muscle protein breakdown by urinary 3-methylhistidine (3MH), muscle soreness, isokinetic muscle strength, muscle glycogen resynthesis, and serum hormones. Untrained males (N=26) underwent a 9-day controlled meat-free diet and 24 hr urine collections. To reduce glycogen, subjects cycled for 40 min at 70% of VO_{2peak} followed by 5 cycling sprints on day 4 evening. On day 5, fasted subjects performed 100 eccentric leg flexions at 120% of 1-RM and drank C (n=8, 1.25 g C/kg), CP (n=9, 0.875 g C/kg, 0.375 g protein/kg), or P (n=9) beverages immediate post-exercise (IPE) and 2 hr later. Muscle biopsies were taken IPE on day 5, and days 6 and 8 mornings. Blood was obtained days 4-10 fasted plus IPE, 3 hr, and 6 hr post-exercise on day 5. At 3 hr on day 5, insulin was higher for CP (24.6 ± 15.5 μIU/ml) and C (17.2 ± 10.9 μIU/ml) than P (5.3 ± 0.4 μIU/ml, $p<.05$). Glycogen was low on day 5, partially recovered on day 6, and normal by day 8 ($p<.01$) with no difference among groups. Isokinetic quadriceps peak torque at 60^{o}/s decreased 24% on day 6 and remained depressed by 21% on d 8 ($p<.01$) for all groups. Soreness peaked on day 7 and CK on day 8 ($p<.01$) with no differences by group. CK increased ($p<.01$) over day 5 (delta CP 24.6 ± 19.1, delta P 39.2 ± 71.6, delta C 70.8 ± 60.4 U/L) and was highest for C ($p<.05$). On day 5, CP had lowest 3MH (193.0 ± 13.8 μmol/d) versus C (251.1 ± 22.5 μmol/d, $p<.05$). Testosterone at 3 hr on day 5 was lower for C (4.2 ± 0.3 ng/ml) and CP (4.3 ± 0.3 ng/ml) versus P (5.1 ± 0.2 ng/ml, $p<.05$). In summary, glycogen, muscle strength and soreness were unaffected by beverage. However, a CP beverage may be beneficial for reducing muscle damage and protein breakdown on the day of eccentric resistance exercise.

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Chapter 1
Introduction

Dynamic muscular contraction can take place when a muscle produces force either by shortening (concentric contraction) or by lengthening (eccentric contraction). It is the eccentric contraction that has been shown to produce the most structural damage to the muscle and subsequent muscle protein breakdown (Evans & Cannon, 1991). Although a good body of research focuses on eccentric contractions obtained during downhill running, an aerobic activity (Cannon et al., 1991; Evans et al., 1986) several research groups have been able elicit muscle damage and protein breakdown following eccentric resistance exercise, an anaerobic activity (Costill et al., 1990; Clarkson, Byrnes, McCormick, Turcotte, & White, 1986; Gibala, MacDougall, Tarnopolsky, Stauber, & Elorriaga, 1995; Nosaka, Clarkson, McGuiggan, & Byrne, 1991).

The damage process may be initiated due to mechanical stress on the muscle fibers during novel eccentric exercise (Evans & Cannon, 1991; Newham, Jones, & Clarkson, 1987). Nonetheless, in the hours following novel eccentric exercise a metabolic cascade of events begins leading to structural damage to the muscle, biochemical evidence of damage and inflammation, decreased muscular function, and associated muscle soreness (Armstrong et al., 1983; Evans, 1991; Evans & Cannon, 1991; Friden et al., 1983; Friden et al., 1988; Newham, McPhail, et al., 1983). Microscopic evaluation of muscle which has undergone eccentric exercise shows evidence of structural damage to its functional units, the sarcomeres, and evidence of swelling (Armstrong, Ogilvie, & Schwane, 1983; Friden, Sjostrom, & Ekblom, 1981; Friden, Sjostrom, & Eckblom, 1983).

Increased levels of creatine kinase (CK), an enzyme released from the damaged muscle, can be detected in the circulation beginning in the first 24 hr following high-force eccentric exercise (Clarkson et al, 1986; Ebbeling & Clarkson, 1989; Nosaka et al., 1991; Nosaka & Clarkson, 1996). Furthermore, increased urinary levels of an amino acid

metabolite from muscle protein breakdown, 3-methylhistidine, can be detected in the days following intense resistance exercise which contains an eccentric component (Hickson & Hinkelmann, 1985; Pivarnik et al., 1989; Roy, Tarnopolsky, MacDougall, Fowles, & Yarasheski, 1997). Also within 24 hr of exercise, there is evidence of an inflammatory response which includes the infiltration of neutrophils and monocytes which release cytokines. In particular, the cytokines interleukin-1β (IL-1 or IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF or TNF-α) which are associated with muscle protein breakdown (Cannon et al., 1991; Evans et al, 1986; Evans & Cannon, 1991; Miles, Clarkson, Keller, & Hackney, 1995).

Impaired muscle function and soreness are also evident in the days following eccentric exercise. For example, maximal muscle force production is impaired immediately following the exercise bout and gradually recovers over approximately one week. Delayed onset muscle soreness (DOMS) typically develops in 24-48 hr following high force eccentric exercise and peaks at about 3 days post-exercise (Clarkson et al., 1986; Clarkson, Nosaka, & Braun, 1992; Nosaka et al., 1991).

In recent years, several research groups have demonstrated impaired skeletal muscle glycogen resynthesis in muscle which has undergone high-force eccentric resistance exercise (Costill et al., 1990; Doyle, Sherman, & Strauss, 1993; Widrick et al., 1992). The impaired resynthesis is evident from 1.5-3 days following the exercise. Since depletion of skeletal muscle glycogen is associated with fatigue and cessation of endurance exercise performance (Conlee, 1987), the impaired resynthesis could also have implications for athletes who need to perform multiple workouts or competitions over that same time period.

Several research groups have examined nutritional interventions following aerobic exercise that contains an eccentric component. For example, Cade et al. (1991) found that a milk-protein supplement consumed following swim training elicited a lower serum CK response compared to a carbohydrate-electrolyte drink or water. Another study

(Nieman, Nehlson-Cannarella, Henson, Utter, & Davis, 1997) reported lower serum IL-6 responses immediately and 1.5 hr following a 2.5 hr treadmill run for runners who ingested 0.25 L carbohydrate solution every 15 min during the run versus those who consumed placebo.

Some research has been done in the area of nutrition for optimal recovery from resistance exercise, but this research has not emphasized or isolated the eccentric component. Chandler, Byrne, Patterson, and Ivy (1994) reported that both carbohydrate and carbohydrate-protein beverages consumed immediately and 1 hr following a resistance circuit training session produced higher serum insulin levels for up to 8 hr when compared to a protein beverage or water. Furthermore, higher serum growth hormone was seen for the carbohydrate-protein beverage at 6 hr following the exercise session. This hormonal environment produced by the carbohydrate-protein beverage was interpreted as favorable for both muscle glycogen and contractile protein synthesis. Another study which examined carbohydrate consumption following a resistance circuit training exercise (Roy et al., 1997) found lower urinary 3-MH excretion for the supplemented subjects versus placebo 24 hr following exercise. Therefore, the possibility exists that post-exercise dietary manipulations may influence the degree of muscle damage from eccentric resistance exercise as well as promote a hormonal environment favorable for muscle glycogen and contractile protein synthesis.

Purpose

The purpose of this study is to examine if a carbohydrate-protein, carbohydrate, or placebo beverage consumed immediately and two hours following eccentric resistance exercise influences:

1. Muscle glycogen resynthesis up to 72 hr post-exercise,
2. Serum levels of hormones associated with muscle glycogen synthesis and muscle protein synthesis (insulin, testosterone, growth hormone),

3. Serum levels of a hormone associated with muscle catabolism and inflammation (cortisol),
4. A marker of skeletal muscle membrane damage (serum creatine kinase) and contractile protein breakdown (urinary 3-methylhistidine), and
5. Quadriceps muscle soreness and isokinetic quadriceps exercise performance.

Significance

This study examines the effects of post-exercise dietary manipulations on recovery from intense eccentric resistance exercise. Very little research has been performed in this area to emphasize eccentric exercise. Although it may not be possible to influence muscle damage caused by the mechanical events of eccentric contractions, it may be possible to influence the metabolic response that begins in the post-exercise period. Therefore, this study intends to clarify metabolic responses to dietary manipulation in the hours and days following an acute bout of intense resistance exercise.

The results of this study are potentially beneficial to athletes who participate in multiple workouts per day (triathletes, football players), those who participate in multiple competitions in one day (wrestlers), and those who participate in competitions over several consecutive days (swimmers, gymnasts). The type of post-exercise beverage consumed by these athletes may promote a hormonal environment more favorable for muscle glycogen and muscle protein synthesis as well as lessen indicators of muscle damage, breakdown, and inflammation.

Hypotheses

The hypotheses of this study were examined in two parts: (a) on the day of eccentric exercise—experimental day 5, and (b) morning values before and following the eccentric resistance exercise. All hypotheses are stated in the alternate form so that direction is specified.

H_{A1}: A carbohydrate-protein beverage, consumed immediately and 2 hr after eccentric resistance exercise, results in lower serum creatine kinase immediately, 3 hr, and 6 hr after eccentric resistance exercise on experimental day 5 when compared to the carbohydrate and placebo beverages. This same hypothesis was examined for the variables serum cortisol and muscle soreness.

H_{A2}: A carbohydrate-protein beverage, consumed immediately and 2 hr after eccentric resistance exercise, results in higher serum insulin immediately, 3 hr, and 6 hr after eccentric resistance exercise on experimental day 5 when compared to the carbohydrate and placebo beverages. This same hypothesis was examined for the variables serum testosterone, serum growth hormone, serum glucose, and muscle soreness.

H_{A3}: A carbohydrate-protein beverage, consumed immediately and 2 hr after eccentric resistance exercise, results in higher skeletal muscle glycogen measured 24 and 72 hr after eccentric resistance exercise when compared to the carbohydrate and placebo beverages.

H_{A4}: A carbohydrate-protein beverage, consumed immediately and 2 hr after eccentric resistance exercise, results in lower morning serum creatine kinase on experimental days 6-10 when compared to the carbohydrate and placebo beverages. This same hypothesis was examined for the variables serum cortisol, urinary 3-methylhistidine, and muscle soreness.

H_{A5}: A carbohydrate-protein beverage, consumed immediately and 2 hr after eccentric resistance exercise, results in higher morning serum testosterone on experimental days 6-10 when compared to the carbohydrate and placebo beverages.

H_{A6}: A carbohydrate-protein beverage, consumed immediately and 2 hr after eccentric resistance exercise, results in higher isokinetic peak muscular torque measured 24, 48, and 72 hr after eccentric resistance exercise when compared to the carbohydrate and placebo beverages. This same hypothesis was examined for the variables work with the maximal isokinetic repetition and total isokinetic work for 5 repetitions.

Delimitations

The following delimitations were established for this study:

1. Subject selection was limited to males age 18-30 years who were present on the Virginia Polytechnic Institute and State University campus or in the Blacksburg, Virginia surrounding area.
2. Subjects were free from chronic disease or orthopedic limitations that would preclude their involvement in strenuous exercise.
3. Subjects were of untrained fitness status with respect to performing no weight training exercise on their legs or running training within the past six months.
4. Subjects did not have a history of lactose intolerance.
5. Although the subjects consumed a controlled diet for 9 days the carbohydrate-protein, carbohydrate, and placebo treatment beverages were given only on day 5 following eccentric resistance exercise.
6. Diet was controlled by having subjects consume prepared meals in a food-preparation classroom or by giving the subjects a boxed meal to consume away from the preparation area.
7. The subjects were not housed on-campus for the experimental period. They returned to their homes after their evening meals and returned the following morning.

Limitations

The following are potential limitations of this study:

1. The results of this study may be generalized to males of similar age and training status as defined by the subject sample.
2. Although practice sessions were given, the subjects' lack of familiarity with exercise training may have influenced their effort during the isokinetic testing, one repetition maximum testing, aerobic cycling, and eccentric resistance exercise.
3. The timing of blood draws was limited to fasting, immediate, 3 hr, and 6 hr post-exercise on day 5. Therefore, the response of serum insulin and serum glucose to

the treatment beverages is limited to these time points. More frequent sampling was not possible due to technician availability, subjects' class or work schedules, and limitations set by the Virginia Polytechnic Institute and State University Institutional Review Board.

4. A dietary baseline period limited to four days and may have affected baseline blood and urine measurements.
5. No biochemical assessments of nutritional status were performed on the subjects prior to the start of the experimental period.

Basic Assumptions

The following assumptions were made in this study:

1. The subjects refrained from any other physical activity during the course of the experimental period.
2. The subjects were honest in reporting their daily dietary intake on the check-off sheets provided to them.
3. The subjects refrained from taking any pain relief, anti-inflammatory medication, or vitamins during the course of the experimental period.
4. Changes in blood, urine, and muscle were due to the exercise treatment and/or treatment beverages and not caused by extraneous environmental factors.
5. All subjects accurately reported their perception of muscle soreness on the 1-10 scale.
6. All subjects gave a maximal effort during the exercise testing protocols (isokinetic exercise performance, VO_{2peak} testing) and training protocols (endurance cycling and sprints, eccentric resistance exercise).

Definition of Terms

Concentric Muscular Contraction. Shortening of a muscle as it develops tension or force.

Eccentric Muscular Contraction. Lengthening of a muscle as it develops tension or force.

One Repetition Maximum (1-RM). The maximum amount of mass that can be lifted for only one time and is considered an indicator of muscle strength.

Peak Oxygen Uptake (VO_{2peak}). The highest level of oxygen use by skeletal muscle, heart, and lungs during an incremental cycling or running exercise protocol to exhaustion. It is considered an indicator of aerobic or cardiorespiratory physical fitness.

Isokinetic Peak Torque. The maximal amount of rotational force produced at a joint when speed of movement is held constant.

Creatine Kinase (CK). An enzyme found in skeletal muscle that is believed to "leak"out of the muscle into the bloodstream in response to damage-producing high-intensity exercise, particularly eccentric muscular contractions.

3-Methylhistidine (3MH). An amino acid, which is a constituent of the actin and myosin contractile components in muscle and is believed to be an indicator of muscle protein breakdown. Since this amino acid cannot be further metabolized or incorporated into new muscle protein, it is excreted and can be detected in the urine (Ballard & Thomas, 1983; Young & Munro, 1978).

Glycogen. The storage form of carbohydrate concentrated in the heart, liver, and skeletal muscle that consists of chains of glucose linked at the 1, 4 and 1, 6 carbon atoms.

Chapter II
Review of Literature

Overview

This chapter will describe muscle damage, its proposed mechanisms, biochemical and functional indicators of muscle damage, and the role of hormones in muscle damage and resynthesis of new muscle protein. Additionally, this chapter will explore muscle glycogen breakdown and resynthesis following resistance exercise, and how nutritional interventions following resistance exercise may promote muscle glycogen resynthesis and synthesis of new muscle protein.

Muscle Damage Following Exercise

Exercise involving novel eccentric, as well as high-intensity concentric (Armstrong et al., 1983; Friden, Seger, & Ekblom, 1988), muscular contractions has been associated with microscopic damage to the skeletal muscle (Armstrong et al., 1983; Friden, 1984; Friden et al., 1981; Friden et al., 1983; Gibala et al., 1995). In both animal and human models, electron microscopy of eccentrically exercised muscle reveals disruption of the sarcomere, the functional unit of the muscle (Armstrong et al., 1983; Friden et al., 1983; Friden, 1984). Much of the disruption is localized to the cytoskeletal material, particularly the Z-lines, which help keep the contractile elements in place (Friden et al., 1983; Friden et al., 1988; Newham, McPhail, Mills, & Edwards, 1983). Microscopy reveals what is referred to Z-line streaming where the Z-lines appear in a zigzag pattern rather than their normal straight line pattern, and Z-line smearing where the Z-lines extend into the I-band contractile filaments (Friden, 1984). This microscopic damage appears to peak about three days following the eccentric exercise. Muscle biopsies taken six days following eccentric exercise have shown virtually no evidence of damage (Friden et al., 1983).

In unexercised control muscle, electron microscopy reveals only about 4-6% of fibers with Z-line streaming (Friden, 1984; Friden et al., 1988). However, muscle biopsies taken 3 days following eccentric exercise reveal Z-line streaming in 28-36% of samples examined (Friden, 1984; Friden et al., 1983). Another finding from electron microscopy is Type II muscle fibers, the fibers associated with higher force production, show more evidence of damage than the Type I muscle fibers associated with high oxidative, or aerobic, capabilities. The ratio of Type II:Type I fibers involved is about 3:1 immediately and 3 days following eccentric exercise. Immediately post-exercise, more Type IIB fibers are affected, but by 3 days almost as many Type IIA fibers as Type IIB fibers show evidence of damage (Friden et al., 1983).

Other indicators of muscle damage seen by electron microscopy include presence of fluid within the muscle cells, presence of vacuoles, and presence of macrophages (Armstrong et al., 1983; Friden et al., 1988; Stauber, Clarkson, Fritz, & Evans, 1990). Necrotic muscle fibers also have been reported, but fiber necrosis appears to be more common in animal models rather than human studies (Armstrong et al., 1983; Friden et al., 1988; Stauber et al., 1990). However, fibers undergoing necrosis may be fibers near the end of their life cycle (Armstrong et al., 1983; Newham et al., 1987).

Interestingly, there are signs of muscle repair that are evident by electron microscopy. At about three days following eccentric exercise, there is evidence of ribosomes that initiate muscle protein synthesis, mononuclear cells which help phagocytize debris (Friden, 1984; Stauber et al., 1990), and evidence of new myofibril development (Armstrong et al., 1983).

In conclusion, muscle that undergoes eccentric exercise has been associated with substantial increases in microscopic evidence of damage to the structural components as well as presence of inflammatory changes and necrotic fibers. After the damage peaks at about 3 days following exercise, signs of muscle repair and regeneration can be apparent.

Mechanisms of Muscle Fiber Injury During Eccentric Exercise

The mechanisms for the muscle damage seen with eccentric exercise remain to be clarified. These hypotheses include free radical-induced damage, temperature increases within the working muscle, increased metabolic waste products, depletion of metabolic substrates such as ATP and glycogen, and mechanical stress to the muscle (Armstrong et al., 1991; Ebbeling & Clarkson, 1989; Evans & Cannon, 1991).

Free-Radicals. Researchers have attempted to link muscle damage to lipid peroxidation from free radicals. These highly-reactive chemical species with unpaired electrons may affect the structural integrity of the muscle cell membrane to cause damage (Davies, Quintanilha. Brooks, & Packer, 1982). A study that examined runners following an 80 km race found a 77% increase in serum malondialdehyde (MDA) levels, which indicated elevated lipid peroxidation. They also observed other indicators of muscle damage post-race such as increased serum CK and increased serum lactate dehyrdrogenase (LDH) (Kanter, Lesmes, Kaminsky, La Ham-Saeger, & Nequin, 1988). On the other hand, the lower metabolic cost, or oxygen consumption, of eccentric exercise has long been established (Asmussen, 1953), so it is plausible that eccentric exercise may not lead to increased free radical production within the muscle. Furthermore, a study which examined muscle MDA following bouts of 80 concentric and eccentric leg extensions reported no significant increases in muscle MDA over time as well as between the concentrically and eccentrically exercised muscles immediately and 2 days following the exercise (Saxton, Donnelly, & Roper, 1994).

Increased Temperature. Elevated body temperature (Kanter et al., 1988), and elevated muscle temperature (Nadel, Bergh, & Saltin, 1972) have also been suggested as a cause of muscle damage from eccentric exercise. Temperature increases do have potential to damage the muscle cell's anatomic and functional components by altering protein structure (Ebbeling & Clarkson, 1989). Temperature increases measured over the quadriceps muscles during eccentric cycling exercise were only slightly elevated (1.2^{o}C) over the concentric cycling exercise (Nadel et al., 1972). This temperature increase may

not have been sufficiently high to induce damage. It is also possible that at similar relative metabolic rates, muscle temperature during eccentric exercise could actually be lower than for concentric exercise (Armstrong et al., 1991).

Waste Products. Metabolic waste products such as lactic acid have been indicated as a cause of muscle damage. Rats that ran downhill on a treadmill demonstrated more microscopic evidence of muscle damage despite comparable LDH production than in rats that ran uphill (Armstrong et al., 1983). In another study, rats performing low intensity treadmill running (30 m/min for 60 min) showed evidence of muscle damage 24-48 hr post-exercise even though no increases in lactate were reported. (Kuipers, Drukker, Frederik, Geurten, & Kranenburg, 1983). Post-exercise lactate levels from this study were 3.24 ± 2.25 mmol/L in the exercised rats, while lactate levels for unexercised control rats were 3.18 ± 1.50 mmol/L. Again, the lower metabolic cost of eccentric exercise (Asmussen, 1953) would not favor increased lactate production. Therefore, it is not likely that lactate production is causally related to muscle damage with brief eccentric exercise although lactic acid may play a role in the fatigue and pain following intense exercise (Cleak & Eston, 1992).

Metabolic Substrates. Depletion of metabolic substrates has been thought to cause muscle damage. Although some researchers have suggested depletion of ATP to be a factor in muscle damage, the lower metabolic cost of eccentric exercise would not indicate a mismatch between ATP demand and production (Armstrong et al., 1991; Asmussen, 1953; Ebbeling & Clarkson, 1989).

Muscle glycogen depletion has been proposed as playing a role in muscle damage. Type II muscle fibers that showed the most microscopic evidence of damage post-exercise also showed evidence of glycogen depletion, though not in all fibers (Friden et al., 1988). Although muscle glycogen was not measured in their study, it is possible glycogen depletion may have played a role in muscle damage as suggested by increased serum CK, LDH, and MDA in runners after an 80 km race (Kanter et al., 1988).

In a study where untrained male subjects performed three 15-min bouts of eccentric cycling exercise at 90%, 80%, and 70% of VO_{2max}, post-exercise muscle glycogen levels were 60% of resting values (O'Reilly et al., 1987). Electron microscopy of the post-exercise sample revealed glycogen depletion, presence of edema, and Z-band streaming. A repeated biopsy sample 10 days later showed muscle glycogen levels to be only 40% of resting values; however, this value was not statistically different from the post-exercise sample. Microscopic analysis of this 10-day biopsy sample indicated the incomplete glycogen resynthesis, presence of inflammatory cells, necrotic fibers, and no signs of fiber regeneration such as presence of ribosomes which would indicate new muscle protein synthesis. Interestingly, all these changes occurred across both Type I and Type II muscle fibers. This study was the first to match glycogen depletion and impaired glycogen resynthesis with microscopic evidence of muscle damage. The authors proposed that structural damage to the muscle cell could have interfered with glucose uptake, thus, the inability to resynthesize glycogen (O'Reilly et al., 1987). Therefore, although ATP depletion may not play a direct role in muscle damage, several studies have indicated that glycogen depletion, particularly in Type II fibers, and muscle damage may be associated.

Mechanical Stress. It has been hypothesized that mechanical stress to the muscle may be responsible for the initial damage seen following eccentric exercise. When the muscle lengthens as it contracts, a decreasing number of actin and myosin cross-bridges occur. Therefore, higher force produced per fiber during eccentric contractions may expose the muscle to excessive loads causing deformations and damage (Armstrong et al., 1991). The Type II muscle fibers may especially be vulnerable to damage following eccentric contractions because of their high-force but easy-fatigue properties (Lieber & Friden, 1988). The relatively larger size and possibly thinner Z-lines of Type II compared to Type I fibers in humans may cause them to be more susceptible to damage (Friden & Lieber, 1992). Yet, electromyography (EMG) during both concentric and eccentric exercise confirmed that muscle electrical activity is lower during submaximal and

maximal eccentric exercise compared to concentric exercise which would mean fewer muscle fibers are recruited with eccentric exercise (Bigland-Ritchie & Woods, 1976; Rogers & Berger, 1974; Newham, Mills, Quigley, & Edwards, 1983). This may be as a result of input from the central nervous system which would allow the muscle to generate more force more efficiently (Stauber, 1989). Overall, these studies would support the hypothesis that fewer muscle fibers are recruited during eccentric work, but these fibers, particularly Type II muscle fibers, produce more force per cross-sectional area.

Studies performed in the early 1980's reported disruption at the Z-lines indicating that this structural component may be a weak link in the cytoskeleton (Armstrong et al., 1983; Friden et al., 1983; Friden, 1984; Friden et al., 1988). More recent studies indicate another cytoskeletal structural component, desmin, may be particularly vulnerable to eccentric contractions (Lieber, Thornell, & Friden, 1996).

Exactly how the mechanical damage to the muscle occurs remains to be clarified, although several interesting and plausible explanations have been offered. For example, when the Type II fibers fatigue during eccentric contractions, they may enter a rigor or high-stiffness state. When these stiffened fibers are further stressed during subsequent eccentric contractions, damage occurs to the myofibrils and cytoskeleton. Therefore, it is possible that damage may occur in the early stages of the exercise protocol (Friden & Lieber, 1992).

This hypothesis was tested in a study that examined changes in desmin following 5, 15, or 30 min of eccentric contractions in rabbit anterior tibialis (TA) and extensor digitorum longus (EDL) muscles. In comparison to isometrically contracted or passively stretched muscles, the EDL fibers that underwent eccentric contraction showed significant disruptions in desmin by 5 minutes of exercise (2.5% of fibers). Both EDL and TA showed disruptions in desmin by 15 min of eccentric exercise (7.4 % for EDL and 4.6% for TA) with this trend continuing after 30 min of exercise (10.4% for EDL and 5.3% for TA). Furthermore, these abnormal fibers were classified as Type II muscle

fibers. The additional forces produced from the eccentric contractions abated at approximately 5 min in all eccentric exercise conditions. These results were interpreted as evidence of structural damage from eccentric exercise occurring within the first 5-15 min of activity, and this damage may have been related to the fatigue of Type II muscle fibers (Lieber et al., 1996).

It is possible that changes in speed of contraction between different sarcomeres may affect the amount of mechanical stress placed on the sarcomere. As the velocity of muscle lengthening increases, the amount of force produced goes up about 10 times that of muscle shortening (Friden & Lieber, 1992). Sarcomere length within the muscle varies approximately 1-5% with the shorter sarcomeres capable of higher shortening velocities concentrated at the ends of the muscle fiber (Lieber & Baskin, 1983). Therefore, as adjacent sarcomeres may experience different ranges of force, the Z-lines may also experience stress imbalances that could cause the damage which leads to Z-line streaming observed by microscopy (Friden & Lieber, 1992).

Another proposed mechanism of mechanical muscle damage involves using a materials science perspective with muscle as a ductile material (can elongate by at least 5%). High forces have the capability to physically damage the muscle and affect its function since the energy absorbed from the muscle as a result of eccentric contractions must either be lost as heat or produce structural deformations to the muscle (Armstrong et al., 1991).

It has been questioned whether muscle damage results from either the initial high-force eccentric contraction or is a cumulative result of multiple eccentric contractions--materials fatigue (Armstrong et al., 1991; Warren, Hayes, Lowe, Prior, & Armstrong, 1993). To investigate this, Warren et al. (1993) examined rat soleus muscles which had undergone 0-10 eccentric contractions. The first contraction was set at 180% isometric tetanic tension (P_0), and 4 min rest was given between subsequent contractions. The results indicated that when muscles underwent less than eight eccentric contractions, no

changes were seen in the injury markers P_0, maximal isometric twitch tension, rate of tension development, rate of relaxation, and muscle stiffness when compared to control muscle. However, for muscles that performed more than eight eccentric contractions, dramatic declines were seen in all these injury parameters. As the number of eccentric contractions increased, LDH activity at 60 min post-exercise significantly increased ($r = .30$) which would suggest damage. However, no changes in total muscle calcium concentration were observed which would suggest loss of muscle cell membrane integrity. Therefore, this study concluded that cumulative materials fatigue, possibly outside the muscle fiber, is a possible mechanism of eccentric exercise-induced muscle damage (Warren et al., 1993).

Overall, the exact mechanisms of the muscle damage process remain unknown. Several hypotheses have been examined, but many of these remain equivocal. It is also possible that muscle damage results from a combination of these factors. In general, researchers generally believe that some sort of initial mechanical stress to the muscle is a plausible cause of muscle damage, but a secondary metabolic response to the mechanical event is also likely (Armstrong et al., 1983; Armstrong et al., 1991; Ebbeling & Clarkson, 1989; Evans & Cannon, 1991; Friden et al., 1988).

Delayed Metabolic Response to Eccentric Exercise

Despite experimental evidence for this mechanically-based interpretation for muscle damage following eccentric exercise, it may not be the entire explanation. Because of the increase in microscopic evidence of muscle damage and presence of inflammatory cells seen in the immediate days following eccentric exercise, it has been hypothesized there is a secondary delayed metabolic response to the initial mechanical event (Armstrong et al., 1983; Cannon et al., 1991; Evans, 1991; Evans & Cannon, 1991; Friden et al., 1983; Friden et al., 1988; Kuipers, 1993; Newham, McPhail, et al., 1983).

The initial damage to the muscle due to the mechanical event may lead to increased mitochondrial, and possibly intracellular, calcium within the muscle (Duan, Delp, Hayes, Delp, & Armstrong, 1990). Damage to a structure such as the sarcolemma would affect its permeability to calcium and lead to calcium entering the muscle down its concentration gradient (Armstrong et al., 1991). Excess calcium may accumulate in the mitochondria and impair resynthesis of ATP which would affect muscle function (Armstrong et al., 1991; Duan et al., 1990; Kuipers, 1993). Increased intracellular calcium may activate proteolytic enzymes that can disrupt the myofilaments. An example of these proteases is calpain, which may play a role in hydrolyzing the myofilaments. Action of calpain may need desmin as a substrate in addition to increased intracellular calcium (Lieber et al., 1996). Lysozomal proteases, which could destroy the milofilaments after the lysozomes undergo mechanical damage, may also be affected by increased intracellular calcium. Since experimental evidence is inconclusive, it is unlikely lysozomal proteases play a large role in damage to the myofilaments following eccentric exercise (Armstrong et al., 1991; Ebbeling & Clarkson, 1989). It is possible that increased intracellular calcium may activate the phospholipase A_2 pathway, where phospholipids from the cell membrane are utilized to synthesize prostaglandins, leukotrienes, and thromboxanes which have been implicated in inflammation (Armstrong et al., 1991; Greenspan & Baxter, 1996).

There is further progression of these metabolic processes to include the acute release of the enzyme creatine kinase (CK) from the muscle into the bloodstream beginning within 24-48 hr post-exercise. (Clarkson et al., 1986; Clarkson & Ebbeling, 1988; Evans et al., 1986; Newham et al., 1987; Nosaka, Clarkson, McGuiggan, & Byrne, 1991; Smith et al., 1994). Also, within 24 hr of muscle injury, an inflammatory response is initiated which includes elevation of neutrophils, monocytes, and cytokines that help to increase protein breakdown by phagocytizing debris. Increased serum levels of the cytokines IL-1, IL-6, and TNF are typically associated with increased muscle protein breakdown (Cannon et al., 1991; Evans et al, 1986; Evans & Cannon, 1991; Friden, 1984; Miles, Clarkson, Keller, & Hackney, 1995; Stauber et al., 1990).

However, not all the responses to eccentric exercise occur acutely. A more delayed response is commonly seen over several days with elevation in the urine of the compound 3-methylhistidine (3MH), which appears from the breakdown of the actin and myosin contractile components in the skeletal muscle (Evans et al., 1986; Hickson & Hinkelmann, 1985; Pivarnik et al., 1989). Although some studies have found increases in 3MH by 3 days post-resistance exercise, which contains an eccentric component, (Pivarnik et al., 1989), Evans et al. (1986) found gradual increases in 3MH which became significant 10 days following one 45 min bout of eccentric cycle exercise.

Delayed onset muscle soreness (DOMS) in the affected muscles, particularly near the tendinous junctions, also occurs within 24-48 hr following eccentric exercise and peaks about 3 days following high-force eccentric exercise (Clarkson et al., 1986; Cleak & Eston, 1992; Nosaka et al., 1991; Smith et al., 1994). Although the timing and pattern of this pain have been correlated to the increase in serum CK, DOMS and CK are probably not causally related since some subjects can report considerable soreness but marginally increased serum CK (Newham et al., 1987). In addition to DOMS, there is evidence of decrease in maximum muscle strength immediately following exercise which persists in the days following eccentric exercise (Evans & Cannon, 1991; Newham et al., 1987; Nosaka & Clarkson, 1996; Clarkson et al., 1992). This strength deficit post-exercise is about 50% of pre-exercise values and gradually recovers by 7-10 days post-exercise (Clarkson et al., 1992; Newham et al., 1987).

Therefore, a secondary delayed metabolic response to eccentric resistance exercise is very likely to occur in response to the initial injury to the muscle. Although the exact mechanism of this response remains unknown, increased intracellular or mitochondrial calcium may play an important role in its initiation. This metabolic response can be tracked by microscopic evidence of inflammatory changes in muscle in the immediate days following eccentric exercise with concomitant increases in serum and urinary biomarkers of damage and inflammation and increases in muscle soreness.

Indicators of Muscle Damage Following Eccentric Resistance Exercise

Creatine Kinase. Creatine kinase (CK) is an enzyme found in both skeletal and cardiac muscle and is released into the bloodstream within 24 hr following injury to the muscle (Clarkson et al., 1986; Clarkson & Ebbeling, 1988; Cleak & Eston, 1991; Evans et al., 1986; Newham et al., 1987; Nosaka et al., 1991; Smith et al., 1994). Again, a proposed mechanism involves increased intracellular calcium following mechanical damage to the muscle cell membrane, causing a leakage of CK, myoglobin, and other enzymes into the plasma (Armstrong et al., 1991; Duan et al., 1990; Ebbeling & Clarkson, 1989; Lieber et al., 1996). Other hypotheses of increased CK following eccentric exercise have been proposed, and they are similar to those hypotheses for the initial muscle damage. One mechanism examines the possibility that free radicals released from the mitochondria may attack the cell membrane and lead to damage (Kanter et al., 1988). Another mechanism involves possible inhibition of the sarcolemma Na^+-K^+-ATPase pump due to the lower metabolic demand of eccentric contractions. This inhibition may cause accumulation of intracellular sodium and water leading to rupture of the cell membrane (Evans & Cannon, 1991; Macknight & Leaf, 1977; Robinson, 1968).

The largest responses in serum CK appear after novel eccentric exercise, particularly in untrained subjects (Clarkson et al., 1986; Newham et al., 1987; Nosaka et al., 1991; Paul et al., 1989; Stauber et al., 1990). Costill et al. (1990) had 8 "normally active" subjects perform 10 sets of 10 repetitions of one-legged eccentric leg flexions at 120% of maximal force (1-repetition maximum, or 1-RM) followed by 60 min of moderate-intensity concentric stationary cycling with both legs at 70% VO_{2max}. There was an increase in CK from a baseline level of 57 U/L to almost 7,000 U/L at 3 days post-exercise. Three subjects had CK levels peak over 8,000 U/L and one had CK levels of 30,000 U/L. From a baseline measurement of approximately 100 U/L, Rodenburg, Bar, and DeBoer (1993) were able elicit CK responses of 3,000 U/L 4 days after 30 min of 120 maximal eccentric arm exercise in untrained male subjects, where the subjects lowered a weight stack from 45^o elbow flexion to 170^o extension. Very high values of serum CK were obtained in six untrained female subjects who performed 70 maximal eccentric arm

contractions at a rate of 1 contraction every 15 s for a total of 20 min of exercise. Peak CK values seen 4-5 days following the exercise ranged from 6,740-24,200 U/L (Nosaka, Clarkson, & Apple, 1992).

These results of Nosaka et al. (1992) are interesting because of the very high CK values obtained and because researchers believe females experience a lower serum CK response than males. This response may be due to increased clearance of CK versus effect of sex hormones since similar levels of microscopic muscle damage were found between the sexes in rats that underwent 1.5 or 2.5 hr of downhill running (Van der Muelen, Kuipers, & Drukker, 1991).

There is evidence of increased CK with trained subjects following long, intense resistance exercise that contains an eccentric component. For example, significantly higher CK values were found for experienced weight trainers at 12 and 24 hr following 3 sets of 6 exercises at 70-80% 1-RM (Paul, DeLany, Snook, Seifert, & Kirby, 1989). Baseline values were slightly higher at 200 U/L than normal 100 U/L (Sigma Chemical Co., St. Louis, MO), CK peaked at about 550 U/L 12 hr post-exercise, and was 400 U/L at 24 hr post-exercise. In this same study, inexperienced weight trainers experienced slightly higher CK at 12 hr (NS compared to the experienced weight trainers), but their 24 hr sample was significantly higher at 650 U/L than the experienced weight trainers.

Another study examined serum CK responses between 10 trained and 10 untrained male subjects performing a bodybuilding-like weight training routine of squats, leg press, leg extension, and lunge on the first day and stiff-legged deadlifts, double leg curls, and single leg curls on the second day (Vincent & Vincent, 1997). All exercises were performed at 12-RM, but the untrained subjects did not perform the stiff-legged deadlift due to possible risk of injury. Serum CK for both groups was elevated 24 hr following the first workout session, but peak CK was seen 4-5 days post-exercise for both groups. The untrained exercisers showed much higher CK at these time points (3,000-3,250 U/L) compared to the trained subjects (500-1,000 U/L).

There have been reports of high-force exercise that emphasized the eccentric component not eliciting a large CK response. In contrast to the study by Costill et al. (1990), a similar study from the same laboratory (Widrick et al., 1992) did not find a significant increase in CK response following eccentric resistance exercise which consisted of 60 one-legged eccentric contractions of the quadriceps at 120% 1-RM. The subjects completed concentric cycling to exhaustion using both legs the evening prior to the eccentric exercise at an intensity of 70% VO_{2max}. However, pre-eccentric exercise CK values were somewhat higher than expected at 227 ± 75 U/L, which may have indicated some muscle damage may have taken place during the aerobic cycling. Doyle et al. (1993) did find a significantly higher CK response when 100 one-legged eccentric quadriceps contractions at 120% 1-RM followed 75 min of two-legged concentric aerobic cycling at 70% $VO_{2max.}$ An additional bout of cycling was completed 2 days after the first bout. In their study, peak CK values 72 hr post-eccentric exercise were moderately elevated to 444 ± 157 U/L from pre-exercise values of 147 ± 23 U/L.

Subject characteristics may have influenced the CK responses observed for the intense resistance exercise protocols as well as some of the high-force eccentric exercise protocols. The studies that used untrained subjects found a much greater magnitude in the CK response in these subjects (Costill et al. 1990; Nosaka et al., 1992; Rodenburg et al., 1993; Vincent & Vincent, 1997). Some studies utilized subjects who were endurance trained but not performing resistance exercise involving their leg musculature and found moderate increases in CK (Doyle et al., 1993; Widrick et al., 1992). The studies that used experienced resistance trainers as subjects reported statistically significant serum CK responses although their responses were less than those of untrained subjects (Paul et al., 1989; Vincent & Vincent, 1997).

Several studies have shown that prior exercise training can elicit an adaptation effect on the CK response (Byrnes et al., 1985; Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1990; Newham et al., 1987). Newham et al. (1987) had 8 subjects who had not

participated in eccentric exercise for at least 3 months undergo 70 repetitions of maximal eccentric arm exercise. Three bouts were performed, each separated by two weeks. After the first bout, CK peaked on day 4 post-exercise at 10,000 U/L, the second bout produced peak CK levels of 330 U/L 2-3 days post-exercise, and the third bout produced peak CK level of 230 U/L 1 day post-exercise. Another study examined the effects of a repeat bout of eccentric arm exercise 5 or 14 days following an initial bout of 70 maximal eccentric arm repetitions found only modest increases in CK after the second bouts (Ebbeling & Clarkson, 1990). In order to examine how long the CK adaptation effect lasts, Nosaka et al. (1991) had subjects repeat 70 maximal eccentric arm repetitions 6 weeks, 10 weeks, and 6 months following the first bout. The initial bouts produced peak serum CK levels of approximately 4,000-6,000 U/L 4-5 days post-exercise. The second bouts at 6 and 10 weeks produced much lower serum CK at 330 U/L and 800 U/L, respectively, although some subjects showed CK levels less than 100 U/L while some showed levels greater than 1,000 U/L. After 6 months following the initial bout, the 6 re-tested subjects had CK from this bout only average 2,500 U/L, and each individual showed a dramatic decrease from their baseline value.

In addition, the intensity for the eccentric exercise to elicit this protective response need not be very high. A small number of repetitions as low as 12 or performance of any unaccustomed activity such as lifting boxes has potential to elicit this CK adaptation response (Clarkson et al., 1992; Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1989). The mechanism for this rapid CK adaptation remains unclear but may include a combination of cellular and neural adaptations. Perhaps fibers that are damaged and necrotic are fibers near the end of their life span that are replaced by stronger fibers (Armstrong et al., 1983). Neural factors may be involved because the adaptation takes place before recovery of other indicators of muscle damage such as muscle strength and ability to fully flex the joint of the involved muscle group (Ebbeling & Clarkson, 1990). Nonetheless, studies have shown a training adaptation to CK develops rapidly following eccentric exercise, even before the muscle fully recovers from other indicators of damage,

and this adaptation can last for up to 6 months (Ebbeling & Clarkson, 1990; Newham et al., 1987; Nosaka et al., 1991).

The use of CK as an indicator of muscle damage has not been without controversy. It has been shown that the CK response is highly variable, and individuals can be classified as high- or low-responders (Clarkson et al., 1986; Clarkson & Ebbeling, 1988). In response to the same exercise protocols, individuals can show virtually no or moderate increases in CK (less than 100 up to 500 U/L) or responses greater than 10,000 U/L. In many of these instances, variable CK responses do not appear to be related to prior training status, amount of muscle soreness observed, or amount of work done during the exercise. When sera from low- and high-responders are mixed, CK values reflect a mixture of the two, and there does not appear to be other factors in the sera from the low-responders which could help explain their response (Clarkson & Ebbeling, 1988). The amount of muscle mass involved in the exercise apparently does not affect CK response—those persons who eccentrically exercised both arms had the similar CK levels as persons who exercised one arm (Nosaka & Clarkson, 1992). Furthermore, the potential for a long-lasting CK adaptation effect with a minimal exercise stimulus has potential to confound interpretation of CK results. Finally, evidence of muscle damage by microscopy can be present without large increases in CK. For example, Armstrong et al. (1983) found no differences in microscopic evidence of muscle damage for rats that ran at either a 16^{o} incline or decline for a total of 90 min exercise, yet CK values for the downhill runners were 300% and 160% higher than the uphill runners immediately and 48 hr post-exercise, respectively.

Overall, increases in serum CK continue to be useful as a biochemical indicator of muscle damage, particularly following novel eccentric exercise in untrained subjects, and there is a good deal of literature detailing its responses. However, researchers may want to take CK responses into account with other indicators of muscle damage since CK can vary among subjects and show a training adaptation response.

3-Methylhistidine. The amino acid 3MH can be detected in the urine from the breakdown of the actin and myosin contractile proteins in muscle. Although certain animals such as the pig can metabolize 3MH to other compounds, in humans its only fate is excretion (Ballard & Tomas, 1983). Urinary 3MH is reported in the literature on an absolute per day basis, relative to body weight or lean body weight, or relative to urinary creatinine, a metabolite of creatine breakdown. Creatinine, like CK, is concentrated in skeletal muscle and is considered a stable indicator of skeletal muscle mass. It is often used to verify completeness of 24 hr urine collections (Heymsfield, Arteaga, McManus, Smith, & Moffitt, 1983).

It has been shown that acute or chronic exercise training can elicit an increase in 3MH excretion. Following 45 min of eccentric cycling exercise at 42% of VO_{2max} in untrained males, Evans et al. (1986) reported gradual increases in 3MH excretion which became significant from baseline 10-12 days following the exercise bout. These values were approximately 25-33% higher than the baseline values. These results were considered indicative of the delayed metabolic response to eccentric exercise. Pivarnik et al. (1989) examined the 3MH excretion following initiation of a 19-day resistance training program in untrained subjects. This program involved 4 sets of 5 exercises at relatively low intensity of 60-70% 1-RM and alternated exercises for the upper and lower body on successive days. It was found that 3MH gradually increased over the training period. These increases became significant 11 days into the training program and represented about a 33% increase over the baseline day 4 values whether they were expressed on an absolute (μmol/day), relative (μmol/kg/day), or per creatinine (μmol 3MH/g creatinine) basis. These data were felt to support increased muscle protein breakdown with resistance training.

A study that examined the effect of resistance training plus with consuming dietary protein at the recommended daily allowance (RDA) or three times the RDA also investigated 3MH excretion over a 28-day resistance training period. The exercise program was similar to that of Pivarnik et al. (1989) in that it involved alternating 6

exercises for the upper and lower body for 3 sets at an intensity of 75-80% 1-RM. When the data were examined in 3-day clusters, it was observed that 3MH gradually increased but more so starting about day 15. Larger increases in 3MH (22%) were observed for both exercise training groups versus the non-training groups (4-16%). It was concluded that resistance training led to gradually increased 3MH excretion despite increased dietary protein intakes (Hickson & Hinkelmann, 1985).

There have been several studies that did not find increased 3MH excretion with resistance exercise training. To examine the acute effects of resistance training exercise on 3MH excretion Hickson et al. (1986) had 10 weight-trained subjects complete 2 resistance exercise protocols while undergoing a meat-free controlled diet for 15 days. The protocols consisted of two 36-min programs to exercise the chest and arms or the back and legs, 3 sets of 6 exercises timed for 30 s of work and 2.5 min rest. Intensity was set at 75-80% of 1-RM, and approximately 6-9 repetitions were completed in the 30 s work period. Subjects were randomly assigned to perform either the chest/arm protocol or the legs/back protocol on experimental day 8 and to perform the other protocol on day 12. Results indicated no changes in 3MH in the days following either exercise protocol regardless if 3MH were expressed on an absolute, per kg body weight, or per g creatinine basis. It was felt isolated bouts of resistance exercise did not affect muscle protein breakdown as measured by 3MH.

Another study also examined the acute effects of resistance training exercise on 3MH excretion (Horswill, Layman, Boileau, Williams, & Massey, 1988). Moderately active subjects (N=9) maintained a self-selected meat free diet for 6 days and performed 9 exercises at intensities of 80%, 60%, and 40% 1-RM for each successive set. Repetitions for all sets were performed to fatigue, and total exercise time was about 2 hr. No changes in 3MH were observed between pre-exercise days and 24 and 48 hr post-exercise when expressed on an absolute basis, per kg lean body weight, and per g creatinine. No changes were seen for urinary hydroxyproline, an amino acid associated with collagen breakdown. However, subjects did report significantly elevated muscle soreness at 24

and 48 hr post-exercise. Results from this study did not support the use of urinary 3MH or hydroxyproline as indicators of muscle damage from resistance exercise.

The effect of a 2 week resistance training program on 3MH in 6 elderly subjects was examined by Yarasheski, Zachwieja, & Bier (1993). The intensity and duration of the resistance training program was 60-75% 1-RM, 2-3 sets for 8-10 repetitions the first week and 75-90% 1-RM, 3-4 sets of 4-8 repetitions the second week. The protocol consisted of alternating upper and lower body exercises performed on isotonic exercise equipment. No changes in 3MH or creatinine were found between the onset and end of the exercise training program. However, quadriceps muscle protein synthesis was increased as measured by infusion of [^{13}C] leucine. It was concluded that the elderly were able to show evidence of new muscle protein synthesis without muscle protein breakdown.

There may be several reasons why unchanged or decreased 3MH responses were seen. The resistance exercise in these studies was only of moderate intensity (Horswill et al., 1988) and duration (Hickson et al., 1986), and both these protocols mixed males and females as subjects. It has been reported that CK responses may be less in females (Van der Muelen et al., 1991), and it is possible that 3MH may follow a similar pattern. The study which used elderly subjects only took 3MH measurements at the baseline and end of the exercise training program (Yarasheski et al., 1993) and may have missed any 3MH changes in the interim. Furthermore, the initial intensity of the exercise program in this study may have been too light at 60-75% 1-RM to influence 3MH.

The role of intensity of resistance exercise on 3MH excretion was examined in a study by Viru and Seli (1992). This study showed that 3MH excretion was from 10-75% higher in the first 6 out of 8 weeks for subjects performing 2 sets of 6 exercises of slow-speed (2-4 s per contraction) total body resistance training, 4 times a week at 70% 1-RM in comparison to subjects who trained at 50% 1-RM. By the eighth week of training, 3MH for the 50% 1-RM group was 11% less than week 1 values, and 14% less for the

70% 1-RM group. Furthermore, the most robust increases in 3MH were observed within 12-24 hr following any of the resistance exercise bouts. All subjects who trained at 70% 1-RM also showed significant increases in cross-sectional thigh area from 1.5-4.0% based on x-ray and circumference measures. This study reported preliminary data that by correcting for meat intake, 3MH is very close to the values reported following a meat-free diet over 5 days. Overall, conclusions from this study make an association between increased muscle cross-sectional area induced by moderately heavy resistance training (70% 1-RM) and elevated levels of 3MH.

There has been some controversy over the use of 3MH as a valid indicator of muscle protein breakdown. High muscle protein turnover in the gastrointestinal (GI) tract and skin may inflate 3MH values obtained from skeletal muscle (Ballard & Tomas, 1983; Hickson et al., 1986). Although some research groups have suggested only 75% of 3MH is derived from skeletal muscle, Ballard and Tomas (1983) estimated that 91% of 3MH excretion is derived from skeletal muscle. These estimations were determined from calculations using a 70 kg reference man (30 kg skeletal muscle), known quantities of 3MH measured in μmol/g protein for various body tissues such as the heart, liver, and lungs, and assuming wet weight of a tissue is 5 times its protein content. According to the calculations, the GI tract and skin contributed 3% each of total 3MH excretion, and other internal organs such as the heart and liver contributed less than 1% each. Further evidence for minimal influence of the GI tract on 3MH was supported by a study by Long et al. (1988) who compared 3MH excretion from normal controls versus patients who had 75% of their GI tract surgically removed. It was found that 3MH excretion was not different between the patients and controls, and these results indicated the GI tract contributes minimally to 3MH excretion.

Overall, urinary 3MH can be used as an indicator of muscle contractile protein breakdown. Most studies involving exercise of sufficient intensity or duration to elicit other indicators of muscle damage have also reported increased 3MH. As is the case with serum CK, validity and reliability of 3MH as an indicator of skeletal muscle protein

breakdown is a source of debate in the literature. Researchers may wish to examine 3MH along with other indicators of muscle damage and muscle protein breakdown.

Impaired Muscle Function. The loss of muscle strength and function have been reported following high-force eccentric resistance exercise. Immediate post-eccentric exercise voluntary maximal muscle force is approximately 50% of pre-exercise levels when subjects perform 70-80 repetitions of eccentric arm exercise (Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1990; Gibala et al., 1995; Newham et al., 1987). There are presently no explanations for this initial deficit in strength, but it may be related to fatigue, mechanical damage, or possibly even a change in neural recruitment to circumvent the fibers that were stressed during exercise (Clarkson et al., 1992). Maximal muscle force recovers much more slowly in the days following eccentric exercise and remains depressed by approximately 30% at 5-7 days post-exercise (Ebbeling & Clarkson, 1990; Newham et al., 1987; Nosaka & Clarkson, 1996). The prolonged strength deficits may be the result of over-stretching the sarcomere due to performing the lengthening eccentric contraction. As a result, there would be less actin-myosin cross bridges available to produce muscular force. However, at the present time there are no experimental data to support this hypothesis (Clarkson et al., 1992; Newham et al., 1987).

Some researchers have hypothesized that losses in muscular force following eccentric exercise are due to delayed onset muscle soreness (Friden, Seger, et al., 1983). Yet, the time courses for muscle strength and muscle soreness after eccentric exercise are different. Muscle strength is lowest immediately and 24 hr post-exercise, but muscle soreness peaks 2-3 days post-exercise (Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1990; Gibala et al., 1995; Newham et al., 1987). Furthermore, deficits in muscle force persist past the time point where muscle soreness disappears. By 10 days post-exercise, muscle soreness has typically abated, but muscle strength can remain depressed by 20% of pre-exercise values (Clarkson et al., 1992). Electrical stimulation of the damaged muscle, which removes voluntary effort, also shows a 50% decrease in force production immediately following eccentric exercise with about 80% recovery of force production 4 days following exercise (Gibala et al., 1995). In summary, the discrepancy

in the time course between impaired muscle force and soreness as well as the evidence for similar force deficits with electrically-stimulated contractions would not support soreness contributing to impaired muscle function (Clarkson et al., 1992).

Other measures of impaired muscle function following high-force eccentric exercise include an increase in the angle of a joint associated with the point of maximal flexion as well as a decrease in the angle of a joint when it is relaxed (Nosaka et al., 1991). The increase in the flexion angle may be related to stretching of sarcomeres which would again prevent the formation of some actin-myosin cross bridges. The time course of the increase in the flexed angle appears to be a reciprocal of the time course for the decrements in maximal force (Clarkson et al., 1992). The decrease in the relaxed angle of a joint may be due to increased intracellular calcium as a result of damage to the sarcoplasmic reticulum (SR). This excess calcium from the SR could cause some actin-myosin cross bridge formation at rest which would lead to the muscle shortening observed in the relaxed state (Armstrong et al., 1991). Although some researchers have proposed muscle swelling contributes to the increased relaxed joint angle, it is not a likely explanation. Once again, the time courses for swelling and relaxed joint angle are different in that peak swelling occurs approximately 5 days following high-force eccentric exercise while the nadir of the relaxed joint angle occurs approximately 3 days post-exercise (Clarkson et al., 1992; Nosaka & Clarkson, 1996).

In conclusion, experimental data indicate impaired muscle function in the days following eccentric exercise. This impairment manifests itself in decreased muscle strength immediately following exercise that gradually recovers over approximately one week, muscle soreness that typically peaks about 3 days following high-force eccentric exercise, and decreased ability to fully flex or fully reflex the joint around the affected muscle group. At the present time mechanisms of impaired muscle function after eccentric exercise remain to be clarified, but overstretching of the muscle fibers, excess calcium, or possibly even swelling and inflammation may play a role.

Influence of Hormones on Muscle Protein Breakdown and Synthesis

Cortisol. Cortisol is the major human glucocorticoid synthesized from cholesterol in the zona fasciculata layer of the adrenal cortex. A stress response in the central nervous system, such as occurring with acute exercise or trauma, stimulates secretion of corticotropin releasing hormone (CRH) from the hypothalamus, which then stimulates secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH causes a rapid synthesis and secretion of cortisol at the adrenal gland. The cortisol is secreted in an unbound state, but only about 10% of cortisol exists free in the circulation. Approximately 75% of cortisol is bound to corticosteroid binding globulin (CBG) while 15% is bound to albumin. Cortisol undergoes a circadian rhythm of secretion with afternoon values (25-100 ng/ml) approximately half of morning values (50-200 ng/ml) (Greenspan & Baxter, 1996).

For cortisol to initiate its biological effects, it first must bind to a specific cytosolic receptor on its target tissue. Once the hormone binds with the receptor, the hormone-receptor complex is translocated to the nucleus where, after binding with the DNA, it stimulates a specific messenger RNA that initiates a specific biologic response (Greenspan & Baxter, 1996). The general action of glucocorticoids such as cortisol is inhibition of DNA, RNA, and protein synthesis as well as accelerating protein catabolism. Therefore, excess glucocorticoids, as a result of a pharmacologic dose or stress response, are associated with muscle protein breakdown (Brillon, Zheng, Campbell, & Matthews, 1995). It is likely that catecholamines secreted during exercise may influence ACTH secretion to affect cortisol synthesis and secretion, but it is also possible that other hormones such as insulin and growth hormone may interact with cortisol following resistance exercise (Kraemer, 1988).

Elevations of serum cortisol below pharmacologic doses may have potential to affect muscle protein breakdown. To evaluate the role of increased cortisol on the rate of appearance of leucine, which would indicate muscle tissue breakdown and gluconeogenesis, 7 male subjects were infused with hydrocortisone sodium succinate at a

rate of 20 ng/kg/min or saline. Each trial was separated by two weeks. Infusion of the hydrocortisone increased plasma cortisol concentrations from 100 ng/ml to 420 ng/ml by the end of 3 hr. Rate of leucine and alanine appearance were measured using isotope infusion for 4 more hours. Glucocorticoid administration led to increased plasma leucine by 70% compared to 30% with saline over the course of the infusion, increased rate of leucine conversion to alanine from 4-8 hr, and no changes in plasma insulin and glucagon. This study concluded that higher plasma cortisol stimulates muscle protein breakdown and may be mediated through the glucose-alanine cycle (Simmons et al., 1984).

There is evidence for both increased and unchanged serum cortisol following resistance training exercise. However, the training status of subjects as well as the intensity, volume, and rest associated with the exercise session most likely play a role in the cortisol response to resistance exercise (Kramer, 1988). Jurimae et al. (1990) found serum cortisol rose 50% in untrained men immediately following a 30 min circuit training exercise consisting of 1 set each of 10 exercises performed for 30 s work and 30 s rest at an intensity of 70% 1-RM. In a study which weight trained and untrained men performed 5 sets of 10 repetitions of squatting exercises with 2.5 min rest between sets, 5 sets of 10 repetitions of partial squats with 1 min rest between sets, and 3 sets of 10 vertical jumps with 1 min rest between sets, serum cortisol was about 50% higher immediately following exercise in both trained and untrained men. Only the values for the untrained men were statistically different from controls that observed the exercise. At 1.5 h post-exercise, serum cortisol remained at the post-exercise level only in the untrained men, and this difference was different from both the trained men and controls (McMillan et al., 1993). Volek et al. (1997) did not find any increases in serum cortisol in weight-trained subjects following 5 sets of 10-RM bench presses and 5 sets of 10 repetitions of a jump squat protocol where subjects performed vertical jumps with 30% 1-RM of their squat resistance. Rest time between each exercise in this protocol was 2 min.

There is some research examining serum cortisol responses following exercise that specifically isolated the eccentric component. When 14 untrained male subjects performed 5 sets of 35 eccentric arm exercise at an intensity of 75% maximal eccentric strength and 2 min rest between sets, no changes in cortisol were seen following exercise (Smith et al., 1994). However, when these subjects were assigned to receive either a 30 min sports massage or a brief application of a lotion and rest for 30 min, a lesser degree of decline in serum cortisol was observed for the massage group. It was felt the massage could have caused a further stress response which lead to the smaller decline in serum cortisol as a possible anti-inflammatory response to the massage. Miles et al. (1995) reported a 200-400% increase in serum cortisol 42 hr post-exercise only in 2 untrained female subjects who demonstrated serum CK responses over 4,000 U/L following 50 repetitions of maximal eccentric arm exercise, and 50 repetitions of knee extension and flexion. Unfortunately, data were collected from a total of 5 female subjects in this study. Their responses to the exercise were quite variable, which makes their findings somewhat difficult to interpret. However, a recent study by Nosaka and Clarkson (1996) did not find any changes in serum cortisol in untrained male subjects either immediately or in fasted samples up to 5 days following 24 repetitions of maximal eccentric arm exercise.

There are data indicating cortisol may play a role in the inflammatory response following eccentric exercise (Gleeson, Blannin, Zhu, Brooks, & Cave, 1995). Six untrained males performed randomized bouts of 30 min of bench stepping with the lead leg alternating every 15 min and 30 min of uphill walking up an 8% grade. The trials were separated by two weeks. Two days following each trial, subjects performed 15 min of concentric cycling at 80% VO_{2max}. The subjects experienced significantly elevated muscle soreness in the calves (4.2 ± 1.6 on 1-10 scale), hamstrings (4.7 ± 1.2), and buttocks (4.9 ± 3.0) 48 hr after the eccentric exercise only, but no changes in soreness were found for the quadriceps. Serum CK was significantly elevated 48 hr post-exercise (323 ± 104 U/L) only following the eccentric exercise. They reported significant increases in serum cortisol and leukocytes 150 min after the concentric cycling in the post-eccentric exercise trial. Unfortunately, the raw data or the percent magnitude of

increase were not reported. However, they interpreted these results that the serum cortisol and leukocyte responses are likely related to each other and may be indicators of exercise stress.

In summary, high levels of serum cortisol are associated with muscle protein breakdown. Cortisol may act on muscle as a result of increased catecholamines during exercise, which can be perceived, as stress. Therefore, intensity, duration, and rest periods during exercise may influence the cortisol response. However, its exact role in muscle hypertrophy and breakdown with exercise training, particularly eccentric exercise, remains to be determined.

Testosterone. Testosterone is a steroid hormone responsible for growth of the male reproduction system at puberty, development and maintenance of male secondary sex characteristics, and skeletal muscle hypertrophy (Hickson, Hidaka, Foster, Falduto, & Chatterhorn, 1994). Less than 1% of testosterone in the circulation is secreted from the zona reticularis of the adrenal cortex, and less than 5% is converted from precursors in peripheral tissues. (Greenspan & Baxter, 1996). Most testosterone is produced in the Leydig cells in the testes in response to gonadotropin releasing hormone (GnRH) from the hypothalamus which stimulates release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary (Chandler et al., 1994; Greenspan & Baxter, 1996).

Only about 2% of testosterone circulates free in the plasma, about 38% is bound to albumin, and about 60% is bound to the glycosylated protein sex hormone-binding globulin (SHBG). Although it is believed that free testosterone is the biologically active form, it is possible that some of the protein-bound testosterone can dissociate from the carrier and enter cells to initiate biologic effects. Once the testosterone enters the target tissue it may be converted by the enzyme 5α-reductase to a more potent form called dihydrotestosterone (Greenspan & Baxter, 1996). It appears that testosterone rather than dihyrotestosterone is preferred to bind with the receptor in skeletal muscle (Kraemer, 1988). Both dihydrotestosterone and testosterone bind to a cytosolic receptor which is

translocated to the nucleus where it is undergoes a transformation to bind with the chromatin. This binding results in the production of a specific mRNA that is transported to the cytoplasm where it initiates new protein synthesis (Griggs et al., 1989).

The exact role of endogenous testosterone on muscle growth following resistance training remains to be clarified. It is assumed that higher circulating levels of testosterone post-exercise or possibly following a training program have potential to facilitate muscular hypertrophy because of its effects during puberty as well as the effects of other anabolic steroids on increasing skeletal muscle mass (Griggs et al., 1989; Hickson et al., 1994). Testosterone may enhance the effects of somatomedins on growth since one study reported testosterone administration alone to hypophysectomized rats did not influence their growth. When growth hormone was administered along with the testosterone, increased body weight occurred (Kawai et al., 1982). Other animal studies reveal testosterone has potential to convert Type IIA muscle fibers to Type IIB fibers in isolated muscle preparations (Kelly, Lyons, Gambki, & Rubenstein, 1985).

There has been some discussion as to whether testosterone acts primarily as an anabolic hormone or an anticatabolic hormone in skeletal muscle since it may compete for binding with glucocorticoid receptors. A study by Capaccio et al. (1987) found that administration of testosterone (40 mg/ kg BW) along with glucocorticoids (20 mg/kg BW) did not prevent glucocorticoid-induced muscle atrophy in a rat model. However, testosterone administered by itself did induce muscle hypertrophy in the same model. Therefore, conclusions from this study are testosterone has minimal affinity for glucocorticoid receptors, and the evidence would not support an anti-catabolic role. In addition, because it is well established that testosterone initiates mRNA synthesis and protein accumulation, further evidence is provided for its anabolic properties (Kraemer, 1988).

Researchers generally believe that resting serum testosterone is not affected by long-term resistance training. However, experimental protocols have been able to elicit

an increase in serum testosterone following heavy resistance exercise. As is similar to the cortisol response, the testosterone response may be related to a threshold intensity of exercise, work volume, or amount of muscle mass exercised (Kraemer, 1988).

The effects of a high volume versus high intensity resistance training program on serum testosterone responses were studied by Kraemer et al. (1991). Recreationally fit male and female subjects performed both protocols in random order with a minimum of 72 h rest between sessions. The first protocol utilized a 5-RM workload for 3-5 sets (depending upon body part exercised) and a 3 min rest period between sets, and the second protocol utilized a 10-RM workload for 3 sets with a 1 min rest period between sets. Both protocols contained the same 8 exercises, 3 for the legs and 5 for the upper body. Total work was higher with the second protocol (calculated by weight x vertical distance moved per repetition x number of repetitions). Blood samples were taken pre-, mid-, immediate post-, and 5, 15, 30, and 60 min post-exercise. Females did not show increases in serum testosterone at any time point in sampling and had values only about 10% of males. Males demonstrated a 15% higher serum testosterone response mid-exercise with the higher volume protocol, but at 60 min post-exercise, the higher intensity protocol elicited about 25% higher serum testosterone.

A limited amount of research has examined dietary influences on serum testosterone responses following resistance exercise. Chandler et al. (1994) had male weight lifters consume either 1.5 g/kg BW carbohydrate, 1.38 g/kg BW protein, 1.06 g/kg BW carbohydrate and 0.41 g/kg BW protein, or water beverages immediately and 2 h following a resistance training circuit with an intensity set at 75% 1-RM. There were 3 exercises for the legs and 5 exercises for the upper body performed for 2 sets of 8-10 repetitions. They reported 25% elevated serum testosterone immediately post-exercise in all subjects. However, 30 min after the first supplement was consumed, serum testosterone fell 15% below pre-exercise values for all treatment groups versus the water control. The subjects who consumed the carbohydrate and protein beverages continued to demonstrate these significantly lower testosterone levels than control from 2-5 hr post-

exercise, while subjects who consumed the carbohydrate-protein beverage showed significantly lower serum testosterone only at 4 hr post-exercise.

Another study (Volek et al., 1997) examined the effect of dietary nutrients on serum cortisol and testosterone responses following a bench press and squat protocol in weight-trained males. The exercise protocol consisted of 5 sets of 10-RM bench pressing and 30% 1-RM jump squats with 2 min rest between sets. Diet records were obtained from the subjects for 17 consecutive days. Blood samples were obtained pre- and 5 min post-exercise while the subjects quietly sat. This protocol significantly elevated serum testosterone 6% following the bench press and 16% following the jump squat compared to pre-exercise values, but there was no effect on serum cortisol values. Interestingly, pre-exercise serum testosterone was significantly negatively correlated with percent daily protein ($r = -.71$), polyunsaturated fatty acid/saturated fatty acid ratio ($r = -0.63$), and protein/CHO ratio ($r = -.59$). Pre-exercise serum testosterone was positively correlated with saturated fatty acid ($r = .77$) and monosaturated fatty acid ($r = .79$), both expressed in g/1,000 kcal/d. This study supported the idea that resting serum testosterone may be related to dietary nutrients in an athletic population. The implications for this study are for athletes who consume a very low fat or high protein diet, which could potentially lower their serum testosterone levels.

In summary, testosterone is a steroid hormone associated with muscular hypertrophy, and heavy resistance exercise may be able elicit large increases in serum testosterone which may facilitate muscular hypertrophy. The serum testosterone responses in the days following eccentric resistance exercise are not known. There is evidence that dietary nutrients may somehow affect the serum testosterone response prior to or following resistance exercise. Further research needs to be performed in order to clarify these relationships.

Growth Hormone. Growth hormone (GH) is a polypeptide hormone (191 amino acids) synthesized and secreted from the anterior pituitary. Secretion is mediated by two

hormones from the hypothalamus, growth hormone releasing hormone (GHRH), also known as somatocrinin, and somatostatin, also known as growth hormone inhibiting hormone (GHIH). Once secreted, GH has a half-life of about 50 min. Growth hormone is also under neural control resulting in an episodic release pattern that typically peaks 1-4 hr following the onset of sleep. Exercise, emotional stress, and other physical stress can lead to GH release as well (Cappon, Ipp, Brasel, & Cooper, 1993; Greenspan & Baxter, 1996).

Macronutrients can affect GH release in that carbohydrate feedings tend to lower GH release (Cappon et al., 1993; Matzen et al., 1990). Conversely, hypoglycemia has been demonstrated to increase GH secretion, even when serum GH is at normal levels. These effects may be mediated through feedback with the ventromedial nucleus in the hypothalamus (Franchimont & Burger, 1975). Studies have indicated protein feedings (Matzen et al., 1990) and ingestion of multiple amino acids tend to increase GH release. Male subjects were fed 1.2 g each L-arginine and L-lysine alone and in combination, and while no elevations in GH were seen when the single amino acids were ingested, a 7-fold increase in GH was observed following the amino acid combination (Isidori, Lomonaco, & Cappa, 1981).

Interactions of GH with other hormones have been noted. Therefore, it is often difficult to distinguish effects due to GH versus its influence on other hormones such as the somatomedins, testosterone, insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-2 (IGF-2) (Florini, 1987; Kraemer, 1988; Yarasheski et al., 1995). However, GH is considered an anabolic hormone since experimental evidence supports its associations with muscle hypertrophy, increased protein and RNA content in muscle, and increased activities of RNA polymerases, and increased ribosomes (Florini, 1987). Approximately 30 years ago, it was first reported that GH administration stimulated amino acid uptake in rat diaphragm muscle (Kostyo, 1968). Follow-up studies indicated RNA synthesis increased in muscles following GH administration in hypophysectomized

rats, although the effect is delayed 18-24 hr. If GH administration is continued, there is increased DNA content in the muscles by 7 days (Kostyo & Reagan, 1976).

As is the case with testosterone, intensity of exercise may play a role on increased serum GH response that is typically seen following acute resistance exercise. Kraemer et al. (1991) found increased serum growth hormone in both male and female subjects following a high volume, 10-RM protocol with 1 min rest between sets in comparison to a high-intensity protocol involving loads of 5-RM and 3 min rest between sets. The GH values observed during high-volume exercise were more than double those reported for the high-intensity protocol. Blood samples taken pre-, mid-, immediate post-, and 5, 15, 30, and 60 min after the high-volume protocol continued to show significant differences from pre-exercise levels as well as from the high-intensity protocol.

There are minimal data regarding effects of long-term resistance training on GH secretion (Kraemer, 1988). A study by McMillan et al. (1993) found a 100% higher GH response immediately following resistance exercise in untrained versus trained subjects although both demonstrated larger responses than non-exercised controls. They felt the higher serum free fatty acids seen in the trained subjects following the exercise session would suggest a GH sensitivity response with training. Other studies have suggested that higher GH leads to increased free fatty acid mobilization, which may be related to the inhibition of glucose by GH (Yarasheski, 1994).

The study by Chandler et al. (1994) examined serum GH responses following the resistance exercise protocol and consumption of different macronutrient beverages immediately and 2 hr post-resistance exercise. Although all groups showed an elevated serum GH response immediately post-exercise, GH responses were elevated 6 hr post-exercise for both the carbohydrate-protein beverage and the carbohydrate beverages compared to protein and water. These results were interpreted as a more favorable environment for protein synthesis with ingestion of carbohydrate-protein beverage. Thus,

post-exercise nutrient consumption has the potential to modify the GH response to resistance exercise.

Insulin. Insulin is a 51-amino acid polypeptide hormone synthesized and secreted from the β cells of the Islets of Langerhans in the pancreas. There are two distinct peptide chains; the A chain contains 21 amino acids, while the B chain contains 30 amino acids (Greenspan & Baxter, 1996). There are small amounts of insulin secreted in the fasted state, but the β cells are sensitive to meals, particularly glucose and amino acids, and secretion is increased at these times (Cappon et al., 1993; Matzen et al., 1990).

There is evidence that consuming protein in combination with carbohydrate can increase the serum insulin response. In a study using diabetic subjects, Nuttall et al. (1984) measured serum insulin responses 0.5 hr and every hour for 5 hr following consumption of 50 g glucose, 50 g protein, and 50 g glucose and 50 g protein. The peak insulin response at 2 hr was significantly higher for the glucose and protein combination in comparison to the other treatments. Spiller et al. (1987) found no differences in the insulin responses of normal healthy adults who consumed beverages containing 15.8, 25.1, 33.6, and 49.9 g protein combined with 58 g carbohydrate. Furthermore, the serum insulin responses were significantly higher in these subjects when they consumed the protein-containing beverages in comparison to when they consumed 58 g of carbohydrate alone.

Insulin is carried free in the bloodstream and binds with specific receptors on target tissues. Its mechanism of action is similar to other protein hormones, but it may interact with a GLUT-4 receptor primarily found in muscle and adipose tissue to enhance glucose uptake (Asp & Richter, 1995; Kirwan et al., 1992).

Many metabolic effects are associated with insulin action. Within the pancreas, insulin inhibits glucagon secretion from the α cells. In the liver, insulin promotes glycogen storage while inhibiting glycogen breakdown as well as facilitating protein,

triglyceride, and very low density lipoprotein synthesis. In muscle, insulin is a potent regulator of glycogen synthetase and facilitates glycogen synthesis by its enhancement of glucose uptake into the cell. In addition, insulin facilitates the entry of amino acids into the muscle cell and inhibits protein breakdown (Bennet, Connacher, Scrimgeour, Jung, & Rennie, 1990; Biolo, Fleming, and Wolfe, 1995; Florini, 1987). It can enhance activity of ribosomes to facilitate protein synthesis (Florini, 1987). In adipose tissue, insulin promotes triglyceride storage into adipocytes and inhibits hormone sensitive lipase, which releases triglycerides from adipocytes (Brillon et al., 1995). Insulin, as is proposed testosterone (Kawai et al., 1982), may have increased anabolic actions due to cross-reactivity with somatomedin receptors (Kraemer, 1988).

During endurance exercise, serum insulin levels typically decrease significantly from resting values. In endurance-trained versus untrained subjects, insulin binding to monocytes, which indicates glucose uptake following feeding, was increased following 3 hr of moderate cycle ergometry exercise. This response can offer rationale for a blunted serum insulin response with training, in accordance with a decreased reliance on carbohydrate for energy during endurance exercise in trained subjects. (Koivisto et al., 1980). Enhanced glucose uptake following exercise would indicate restoration of muscle glyocogen.

In studies which examine serum insulin responses following resistance exercise, there is generally no or minimal (non-significant) decline in the serum insulin response (Chandler et al., 1994; Roy et al., 1997). Another study (McMillan et al., 1993) evaluated serum insulin responses in the hours following a resistance training session. Serum insulin levels were about 80% depressed in trained and about 40% depressed in untrained lifters in comparison to controls 5 hr following exercise. Even in response to a meal one hour post-exercise, the serum insulin remained blunted in these two groups. This study suggests that resistance exercise may have an extended depressive effect on the serum insulin response in the post-exercise period. This blunted insulin response with resistance exercise may illustrate the potential increased insulin sensitivity with resistance training

as is seen with aerobic training. It is possible lower glycogen levels may have facilitated glucose uptake post-exercise, leading to the diminished insulin response.

There is some experimental evidence that eccentric muscular contractions may induce a transient insulin resistance in the days following the exercise. Kirwan et al. (1992) studied untrained males following 30 min of downhill running, 30 min of cycling, and under control conditions. Serum CK and muscle soreness were significantly increased 48-72 hr following the downhill running only. Forty-eight hours following each exercise session, the subjects underwent a euglycemic clamp procedure where serum glucose was maintained at about 88 mg/dl for 2 hr before a 2 hr infusion of insulin at approximately 35 μU/ml. Glucose disposal was significantly lower in the final 30 min of the clamp procedure following the eccentric exercise. It was concluded that the eccentric exercise induced a temporary insulin resistance, and potential mechanisms included either decreased insulin binding to receptors or changes in the GLUT-4 protein concentration in the muscle.

Another study (Asp & Richter, 1995) examined insulin action and glucose transport in a rat model using electrically stimulated maximal eccentric contractions, 4 sets of 10 repetitions with 1 min rest between sets, in the red and white gastrocnemius and soleus muscles. Two days following exercise, the rats were sacrificed, and the muscles were perfused with 0, 200, or 20,000 μU/ml insulin. Results from this study indicated that the white gastrocnemius muscle had impaired glucose transport at insulin concentrations of 200 and 20,000 μU/ml, while the red gastrocnemius demonstrated impaired glucose transport only at 20,000 μU/ml insulin. The soleus muscle did not show any glucose impairment. This may be because the soleus muscle did not demonstrate any glycogen depletion following the exercise protocol. Previous researchers have demonstrated glycogen depletion in fast-twitch fibers following eccentric exercise (Friden et al., 1983). It was hypothesized the impairment in glucose transport and transient insulin resistance was likely related to decreased GLUT4 protein content in the muscles following eccentric exercise.

In summary, although serum insulin generally show no changes in the immediate time period pre- to post- resistance exercise, there is potential for resistance exercise to induce increased insulin sensitivity, a response typically seen following aerobic exercise training. In addition, there is evidence for eccentric exercise to induce a temporary insulin resistance 48 hr following exercise. This insulin resistance could interfere with the muscle's ability to resynthesize glycogen and synthesize new muscle protein.

Overall conclusions from the above studies indicate hormonal responses of some hormones such as testosterone, cortisol, and growth hormone may be influenced by the intensity, volume, or duration of an exercise protocol. The implications for these elevated hormones post-exercise on new muscle protein synthesis remain to be identified. On the other hand, the role of insulin in facilitating muscle glycogen and muscle protein synthesis is well characterized. Optimal serum insulin levels following eccentric resistance exercise may be critical to attenuating indicators of muscle damage and enhancing muscle glycogen and protein synthesis.

Nutritional Interventions to Attenuate Inflammation, Muscle Protein Breakdown, and Muscle Damage Following Exercise

There has been some research to examine whether nutritional interventions may lessen indicators of muscle damage, contractile protein breakdown, and inflammation following exercise. These studies have focused on nutritional supplements consumed either prior to or following exercise.

Cannon et al. (1991) had sedentary males take Vitamin E supplements (800 IU/day) or placebo for 48 days to examine if Vitamin E supplements could play a role as an antioxidant in reducing effects of inflammation seen after downhill running. At the end of the supplementation period, the subjects performed 3 bouts of downhill running (-16% incline) at 75% of previously-determined maximum heart rate. Each bout lasted 15

min and was separated by 5 min rest. At 6 hr post-exercise, most plasma IL-1 was below detectable levels of the assay, but only subjects from the placebo group demonstrated detectable levels of IL-1. When in-vitro cytokine secretion by monocytes was examined, IL-6 secretion was significantly less by 50% for the supplemented subjects versus placebo over the 12 days following the exercise. At 24 hr post-exercise, IL-1 secretion was elevated 154% in the placebo versus supplemented subjects. No other changes in IL-1 secretion were observed, but there was a numerical trend for the supplemented subjects to show a decreased IL-1 response over the 12 days post-exercise. Therefore, this study concluded Vitamin E may have potential to affect the role of cytokines in the delayed metabolic response following eccentric exercise.

The effect of carbohydrate consumption on the cytokine response to running was examined in a study by Nieman et al. (1997). Trained male marathon runners were given 0.75 L carbohydrate or placebo 15 min prior to exercise and consumed 0.25 L of the same beverage every 15 min during a 2.5 hr treadmill run at 75-80% VO_{2max}. To examine the inflammatory response to this treatment regimen, blood samples were drawn pre-exercise, immediately, and 1.5, 3, and 6 hr post-exercise. It was reported that serum IL-6 was less in the CHO supplemented group immediately and 1.5 hr post-exercise. No group by time interactions were seen in serum IL-1β or IL-2, other cytokine indicators of the inflammatory response. Serum IL-1 receptor agonist (IL-1ra), which is synthesized to block the effects of IL-1 (Dinarello, 1994), increased significantly in the placebo group compared to the carbohydrate group, which meant less of the IL-1ra and more of the IL-1 occupied IL-1 receptors in the placebo group. In addition, serum cortisol levels were highest for the placebo group immediately post-exercise, but, unfortunately, neither the data nor the magnitude of change were reported. Significant positive correlations were found immediately post-exercise for both serum cortisol and IL-6 with IL-1ra ($r = .54$), while significant negative correlations were found for immediate post-exercise serum cortisol and glucose ($r = -.67$). This study supports the potential benefits of a carbohydrate beverage to lessen indicators of the inflammatory response following

intense aerobic exercise. Therefore, the potential exists for a carbohydrate beverage consumed during or following exercise to lessen indicators of muscle damage as well.

This potential for a carbohydrate supplement to reduce indicators of muscle protein breakdown and its potential to affect muscle protein synthesis was evaluated in a recent study by Roy et al. (1997). They examined the effect of 1 g/kg BW carbohydrate supplementation consumed immediately and 1 hr following resistance exercise on 3MH excretion and fractional rate of muscle protein synthesis in 8 weight-trained males. The exercise protocol consisted of 4 sets each, 8-10 repetitions of unilateral leg press and leg extension resistance exercise at an intensity of 85% 1-RM. Subjects were given a controlled, meat-free diet for 3 days prior to the exercise, and they collected all urine from 12 hr pre- to 12 hr post-exercise. To evaluate fractional muscle protein synthesis rate, subjects underwent a 10 hr infusion of L-[1-^{13}C] leucine beginning 90 min prior to exercise. Muscle biopsies from the exercised vastus lateralis and unexercised control leg were taken immediately and 5 hr post-exercise for evaluation of fractional muscle protein synthesis rate.

The carbohydrate supplemented group had significantly less 3MH excretion compared to placebo (110.4 $\pm$ 3.62 μmol/g creatinine for carbohydrate and 120.14 $\pm$ 5.82 μmol/g creatinine for placebo). A similar pattern was seen for urine urea nitrogen over the same time period, where a significant 16% decrease was reported in the carbohydrate group versus placebo. There were no changes in urinary creatinine excretion between groups. Fractional muscle protein synthesis rate was elevated 36.1% in the carbohydrate and 6.3% in the placebo condition, but the differences were nonsignificant. Serum insulin was significantly higher for the carbohydrate beverage at 0.5, 0.75, 1.25, 1.5, and 2 hr post-exercise, which is likely related to the timing of the beverage immediately and 1 hr post-exercise. In addition, the areas under the curve for serum insulin and glucose 2.5 hr following exercise were significantly higher for carbohydrate versus placebo (300% higher for insulin, 20% higher for glucose). Therefore, it may be possible that a

nutritional intervention may attenuate muscle protein breakdown by 3MH and facilitate recovery following high-intensity resistance exercise (Roy et al, 1997).

Cade et al. (1991) examined if manipulating macronutrient content of nutritional supplements could affect the CK and serum lactate dehydrogenase (LDH) responses in intercollegiate swimmers during their training season. In the first part of the study, swimmers underwent an 8 week training program consisting of two 2 hr sessions per day. The first 4 weeks were considered a baseline period where all swimmers drank water before and after their training. Next, they were divided into four groups. Two groups were assigned to drink either water or 500 ml of a glucose-electrolyte solution (GES) (6% glucose plus sodium, potassium, chloride, and phosphate) before exercise and 45 and 70 min post-exercise. After 1 week, their training intensity was increased by 10% and remained at this level for the final 3 weeks of the training program. No differences in serum CK were observed between the groups during the baseline period and during the first week of supplementation, although CK was approximately 300 U/L for both groups. When the intensity was increased, CK for the group that drank water significantly increased 72% to 500 U/L and gradually declined over the final 3 weeks to 400 U/L. Over the final 3 weeks of training, CK for the GES group gradually declined to 200 U/L.

In the second phase of the study, the same swimmers underwent another training program with the first 4 weeks as baseline. Baseline serum CK was about 200 U/L. Each of the 4 groups was assigned to one of the following conditions: (a) 500 ml water before and 1000 ml during exercise followed by 500 ml of a 16% sucrose drink immediately post-exercise (IPE), (b) 500 ml water before and 1000 ml during exercise followed by 250-500 ml of a milk-protein supplement IPE that contained 15 g protein and 16% sugar IPE, (c) 500 ml GES before and 1000 ml during exercise followed by 500 ml of the sucrose drink IPE, and (d) 500 ml GES before and 1000 ml during exercise followed by the milk-protein supplement IPE. Exercise intensity remained constant during the first week of supplementation. Intensity was gradually increased so that by the end of 6 more weeks a 25% increase in intensity occurred. When intensity was initially increased, the

group that consumed water and sucrose demonstrated a significant 20% increase in CK over the other groups. Their CK slowly declined but rose again to the same level in the final week of training. The other groups experienced a gradual drop in CK during the rest of the training, but the lowest CK values were observed for the group that consumed the GES and milk-protein supplement combination.

Follow-up measurements within the Cade et al. (1991) study examined CK and LDH responses over 22 hr following a training workout in 8 Olympic swimmers. The interventions were assigned each week as in the following order: (a) 500 ml water before and during training and 500 ml of the 16% sucrose solution IPE, (b) 500 ml GES before and during training and 500 ml of 16% sucrose solution IPE, (c) water before and during training and 250-500 ml of the milk-protein supplement IPE, and (d) 500 ml GES before and during training and 250-500 ml of the milk-protein supplement following exercise. It was found that CK increased 40% less and LDH levels increased 10% less when the glucose-electrolyte solution was consumed prior to exercise in comparison to water. At 22 hr post-exercise both CK and LDH were at or below pre-exercise values when the milk-protein supplement was consumed following exercise.

It was hypothesized by Cade et al. (1991) that the milk-protein supplement was providing amino acids needed to resynthesize catabolized proteins and, thus, allowed for a more rapid recovery. They also felt the glucose-electrolyte solution provided a high carbohydrate load to the muscle to spare muscle protein during exercise and that the electrolytes helped maintain optimal extracellular fluid volume. Unfortunately, other indicators of muscle damage to help support their findings, such as 3MH, were not measured in this study. No description of energy from each supplement was provided, but it is unlikely the treatment interventions were isocaloric. Interpretation of their results is somewhat difficult because the volume of the milk-protein beverage ranged from 250-500 ml (with no rationale for this variation given) and non-randomization of treatment interventions in the follow-up study cannot rule out an order effect.

Chandler et al. (1994) examined the influence macronutrient composition of post-exercise nutritional supplements on indicators of recovery from resistance training exercise. Weight-trained male subjects consumed carbohydrate, carbohydrate-protein, protein, or water beverages immediately and 2 hr following a whole-body resistance training circuit. This circuit consisted of 3 exercises for the legs and 5 exercises for the upper body performed for 2 sets of 8-10 repetitions at an intensity corresponding to 75% 1-RM. Blood samples were obtained 30 min and 1, 2, 3, 4, 5, 6, and 8 hr post-exercise. The serum insulin was significantly higher by 200% for both the carbohydrate and carbohydrate-protein beverages at 30 min and 1 hr post-exercise and was significantly higher (30%) for the carbohydrate-protein beverage at 8 hr post-exercise. Although no measures of protein synthesis, protein breakdown, or muscle glycogen levels were evaluated, this study suggested a carbohydrate-protein beverage consumed following resistance exercise could allow for increased levels of hormones associated with muscle protein as well as muscle glycogen synthesis.

Overall conclusions from the above studies indicate the potential for nutritional interventions to attenuate indicators of inflammation, muscle damage, and muscle protein breakdown following intense exercise as well as promoting a hormonal environment favorable for new muscle protein synthesis. Although the Cannon et al. (1991) study specifically emphasized eccentric exercise, the rationale from the other studies can be extended to study nutritional interventions following exercise that isolates the eccentric component.

Glycogen Breakdown and Resynthesis Following Resistance Exercise

Although the rate of muscle glycogen utilization is higher with resistance exercise in comparison with sustained aerobic exercise, the overall change in muscle glycogen levels is less with resistance exercise due to its briefer duration. Following 5 sets of front squats, back squats, leg extensions, and leg curls performed for 6-12 repetitions each set, bodybuilders showed a significant 40% decrease in skeletal muscle glycogen from 160 ± 20 to 118 ± 24 mmol/kg wet weight (ww) (Tesch, Colliander, & Kaiser, 1986). When

non-weight-trained subjects performed multiple sets of 6 single-leg extensions at 70% 1-RM until exhaustion, post-exercise glycogen levels were decreased about 40% from pre-exercise values (Pascoe, Costill, Fink, Robergs, & Zachwieja, 1993). Roy & Tarnopolsky (1998) also saw a 40% decline in skeletal muscle glycogen when resistance trainers performed 9 exercises for 3 sets of 10 repetitions at an intensity of 80% 1-RM.

Although athletes are generally given the recommendation to consume a high-carbohydrate diet to ensure that glycogen stores are maximized prior to exercise, research has been concentrated on timing, amount, and composition of supplements following exercise. Much of this literature focuses on supplements consumed following sustained aerobic exercise. In one study, trained cyclists completed three 2 hr bouts of cycling, alternating intensity every 15 min at 62% and 75% VO_{2max} (Ivy, Lee, Broznick, & Reed, 1988). Immediately following exercise and 2 hr later, they were assigned to receive water, 1.5 g/kg BW glucose polymer (LOW), or 3.0 g/kg BW glucose polymer (HIGH). The subjects also were given a controlled diet for 3 days prior to data collection that consisted of 50% carbohydrate, 35% fat, and 15% protein. Muscle biopsies were taken immediately, 2, and 4 hr post-exercise. Post-exercise glycogen was similarly low across all groups at about 35 mmol/kg ww. Both carbohydrate treatments resulted in similar levels of glycogen resynthesis at 2 hr and 4 hr post-exercise that were different from placebo. The amount of storage at 2 hr for HIGH was 5.8 ± 0.7 mmol/kg ww and 5.2 ± 0.9 mmol/kg ww for LOW. Glycogen storage from 2-4 hr post-exercise followed a similar pattern for both HIGH and LOW, but was about 20% less than in the previous 2 hr. This study concluded that 1.5 g carbohydrate/kg BW consumed immediately and 2 hr following aerobic exercise is adequate to promote muscle glycogen storage when compared to double the amount.

Another research group (Zawadzki et al., 1992) examined post-exercise macronutrient manipulations following 2 hr of cycling where intensity increased from 50-85% VO_{2max}. The trained cyclists who served as subjects were given supplements with 40.7 protein, 112 g carbohydrate, or 112 g carbohydrate plus 40.7 g protein immediately

and 2 hr post-exercise. Thus, the nutritional interventions were not isocaloric. Results indicated glycogen resynthesis for the carbohydrate-protein beverage was 40% increased over the carbohydrate and 300% increased over the protein beverages. This higher glycogen response for the carbohydrate-protein beverage was attributed to a higher insulin response seen 3 hr post-exercise. Because the carbohydrate-protein beverage contained one-third more energy than the carbohydrate beverage, the energy difference may have influenced the insulin response as well as glycogen resynthesis. However, future research may more clearly define the role of carbohydrate-protein beverages in glycogen resynthesis.

Several studies have evaluated the effect of a dietary intervention on glycogen resynthesis following concentric resistance exercise. Pascoe et al. (1993) evaluated consumption of a carbohydrate beverage (1.5 g/kg BW) or water immediately and 1 hr post leg extension exercise (6 repetitions 70% 1-RM until 50% of full extension was no longer possible) in subjects who were not currently weight training. A controlled diet (5 g carbohydrate/kg BW) was given 2 days prior to each trial. Post-exercise muscle glycogen was about 40% decreased in both carbohydrate and placebo conditions. It was found that there were no differences between trials in glycogen content of the vastus lateralis at 2 hr post-exercise, but the carbohydrate beverage was superior at restoring muscle glycogen at 6 hr post-exercise. At 6 hr post-exercise, glycogen in the carbohydrate condition was 91% of pre-exercise values, but glycogen in the placebo condition remained at the same level it was at 2 hr post-exercise—about 100 mmol/kg ww. Serum insulin and glucose were significantly higher up to 240 min post-exercise in the carbohydrate condition compared to placebo. Therefore, consumption of a carbohydrate supplement immediately and 1 hr following resistance exercise can restore skeletal muscle glycogen to near pre-exercise levels by 6 hr post-exercise.

To examine the glycogen resynthesis effects of isocaloric carbohydrate-protein-fat (66% carbohydrate, 22% protein, 10% fat) and carbohydrate supplements consumed following resistance training exercise, Roy & Tarnopolsky (1998) gave 1 g/kg BW of

either supplement or placebo to trained males immediately and 1 h following a whole body circuit training session (3 sets of 9 exercises at 80% 1-RM). A controlled isocaloric and isonitrogenous diet was given for 3 days prior to each exercise session. In addition, the total energy for each exercise session day was equivalent between trials. Both supplements resulted in comparably elevated serum insulin and glucose responses over 2.5 hr in the post-exercise period when compared to placebo. During this same time frame, the area under the curve for insulin was 300% higher than placebo. The area under the curve for serum glucose was 30% higher and similar for both treatment beverages versus placebo. As a result, the treatment beverages had comparably increased levels of glycogen resynthesis at 4 hr post-exercise when compared to placebo. Conclusions from this study show that an isocaloric carbohydrate-protein-fat beverage demonstrates similar serum insulin, serum glucose, and skeletal muscle glycogen resynthesis as a carbohydrate beverage consumed following resistance exercise.

Several research groups have reported impairment of glycogen resynthesis following eccentric resistance exercise. Costill et al. (1990) had untrained male subjects perform 100 one-legged eccentric flexions then perform 1 hr of concentric cycling with both legs at an intensity of 60% VO_{2max}. Subjects were given either a low (41%) or high (76%) carbohydrate diet for 3 days following the exercise bout. All diets averaged 40 kcal/kg BW/day. Surprisingly, the concentrically exercised leg showed significantly lower post-exercise glycogen that may have been due to the subjects favoring the eccentrically exercised leg during the cycling exercise. At 24 hr post-exercise, there was no difference between exercise and diet conditions with respect to glycogen, but the eccentric exercise conditions had a trend towards less glycogen resynthesis. There was significantly less glycogen (33%) in the eccentrically-exercised muscle 72 hr post-exercise regardless of dietary carbohydrate intake. Although the study concluded that glycogen resynthesis is impaired with eccentric exercise, sufficient amounts of carbohydrate may be able to enhance muscle glycogen storage.

A follow-up study from the same laboratory utilized endurance-trained subjects who exercised to exhaustion on a cycle ergometer at 70% VO_{2max} (Widrick et al., 1992). The following morning they performed multiple sets of 6 repetitions of one-legged eccentric resistance exercise at 120% 1-RM until exhaustion. In order to keep muscle glycogen low until the eccentric exercise the following morning, subjects were given a low carbohydrate snack containing less than 15 g carbohydrate following the cycling exercise. All subjects consumed a high-carbohydrate diet (7 g carbohydrate/kg BW) following the eccentric exercise bout. Muscle biopsies were obtained immediately, 24 hr, and 48 hr post-eccentric exercise.

The amount of eccentric force was decreased about 20% from the first to last set of exercise, angular velocity of contractions was increased about 40% from the first to last set of exercise, and the time of the eccentric contraction decreased 40% from the first to last set. There were no differences in glycogen content between the eccentric and concentrically exercised legs post-exercise. Both legs were comparably low in glycogen at approximately 40 mmol/kg ww. There were no differences in glycogen content between the exercised leg and the non-exercised control leg at 6 hr post-exercise, and glycogen levels had approximately doubled in that time period. However, by 24 hr post-eccentric exercise, the eccentrically exercised leg showed 15% less glycogen than the concentrically exercised leg, and this difference was 24% less by 72 h post-exercise. Electron microscopy of the muscle biopsies at 6 hr post-exercise showed changes in the eccentrically exercise muscle that included some swelling in the endomysial region. Mononuclear cells were visible by 24 hr, and by 72 hr these mononuclear cells appeared to be engulfing necrotic muscle fibers. Apparently, only a small number of fibers underwent these changes, but the amount was not specified. No microscopic findings of muscle damage were seen in the fibers that only underwent concentric exercise. This study supported earlier findings of impaired glycogen resynthesis following eccentric resistance exercise. They felt the apparent impairment in glycogen resynthesis takes place after 6 hr post-exercise so that by 24 hr post-exercise significant impairment can be observed (Widrick et al., 1992).

The relationship between eccentric resistance exercise and impaired glycogen resynthesis was further examined in a study by Doyle et al. (1993). Moderately-trained males who had not performed resistance exercise for 6 months served as subjects. These subjects were maintained on a moderate carbohydrate diet from exchange lists (45% carbohydrate, 40% fat, and 15% protein) beginning 2 days before the experimental period. In order to reduce muscle glycogen, these subjects performed concentric cycling for 75 min at 70% VO_{2max} on experimental day 1 followed by 5 1-min maximal cycling sprints at 100% VO_{2max}. Immediately following the cycling bout on day 1, subjects performed 100 eccentric and concentric leg extensions. The concentric exercise was carried out on an isokinetic dynamometer at a speed of 180^{o}/s, and the eccentric leg extensions were performed on an isotonic exercise station at an intensity of 120% 1-RM. For the eccentric exercise, subjects lowered the weight stack for a 4 s count while research assistants raised it. The subjects repeated the aerobic cycling protocol on experimental day 3. Muscle biopsies were taken from the vastus lateralis 15 min and 4 hr following exercise on days 1 and 3. On days 1 and 3, subjects consumed 0.4 g/kg BW carbohydrate every 15 min for 4 hr.

Post-exercise muscle glycogen on days 1 and 3 were not different between the concentric and eccentrically exercised legs. Day 1 glycogen post-exercise was 33.7 ± 4.1 mmol/kg ww for the concentric condition and 34.4 ± 4.5 mmol/kg ww for the eccentric condition. Rates of resynthesis on day 1 were not significantly different at 9.92 ± 0.73 mmol/kg ww/hr for concentric and 9.08 ± 0.70 mmol/kg ww/hr for eccentric. Day 3 post-exercise glycogen was 45.3 ± 5.2 mmol/kg ww for concentric and 47.5 ± 4.2 for eccentric. However, the rate of glycogen resynthesis was reduced 25% on the 3rd day in the eccentrically exercised muscle at 8.14 ± 0.68 mmol/kg ww/hr compared to 10.97 ± 1.02 mmol/kg ww/hr in the concentric condition. These results were in spite of similar serum insulin and glucose concentrations observed for 4 hr post-exercise on experimental days 1 and 3. Overall conclusions from this study indicate impairment in muscle glycogen resynthesis 48 hr following eccentric resistance exercise. In comparison to concentric

exercise, this impairment of glycogen resynthesis with eccentric resistance exercise is despite similar levels of serum insulin and glucose observed under identical muscle glycogen depletion cycling protocols and post-exercise carbohydrate consumption. Therefore, it was hypothesized insulin action and glucose transport at the muscle cell membrane level may be responsible for impairment of glycogen resynthesis following eccentric resistance exercise.

One proposed mechanism for the delay in muscle glycogen resynthesis following eccentric exercise is that an increase in macrophages, leukocytes, and other cells responsible for the inflammatory process may utilize glucose which would otherwise be available for glycogen synthesis (Costill, 1990; Robergs, 1991). Another proposed mechanism involves impaired glucose uptake by damaged muscle following eccentric muscular contractions (Kirwan et al., 1992; O'Reilly et al., 1987). It is possible that changes in a protein found in the muscle cell membrane, GLUT4, influences glucose transport into the cell following eccentric exercise (Asp, Daugaard, & Richter, 1995). Untrained males who performed four 5-min bouts of maximal eccentric cycling did not demonstrate changes in GLUT4 concentration between the exercised and control muscles immediately post-exercise. Skeletal muscle glycogen decreased 17% following exercise. At 24 and 48 hr following exercise, the GLUT4 concentrations in the exercised muscle were only 68 ± 10 % and 64 ± 10% of those in control muscle. Glycogen content in the exercised muscle remained about 20% less than control. All values for GLUT4 and glycogen were not different from control at 4 and 7 days post-exercise. This study concluded the decreased number of GLUT4 proteins observed following eccentric exercise may contribute to the impaired glycogen resynthesis observed in the days following exercise.

Overall, findings from the above studies indicate muscle glycogen levels can be significantly decreased by resistance training, albeit the magnitude is less than that of aerobic exercise. Furthermore, supplementation with carbohydrate or carbohydrate-protein beverages immediately and 1-2 hr following resistance exercise results in similar

patterns of glycogen resynthesis as observed following exhaustive aerobic exercise. These results may be influenced by comparably high serum insulin levels observed for carbohydrate and carbohydrate-protein beverages in the immediate hours post-exercise.

Moreover, several studies imply glycogen resynthesis is impaired from 24-72 hr following high-force eccentric resistance exercise. One of these studies (Costill et al., 1990) suggested that perhaps high carbohydrate consumption post-exercise could enhance glycogen resynthesis following eccentric resistance exercise. The timing and composition of post-eccentric exercise nutritional supplements remain to be determined.

Summary

Eccentric muscle contractions are associated with the most structural damage to the muscle as observed by microscopy. Signs of structural damage to the muscle fiber increase over about three days following exercise, after which signs of muscle repair and regeneration can be observed (Armstrong et al., 1983; Friden et al., 1983). Although the exact mechanism of damage remains to be clarified, there is a general consensus among researchers that some sort of mechanical damage to the muscle fiber is likely involved (Armstrong et al., 1991; Friden & Lieber, 1992; Warren et al., 1993).

A secondary delayed metabolic response to the initial mechanical event is plausible. This metabolic response can be visually observed by the infiltration of inflammatory cells in the region of the damaged muscle fibers as well as by biochemical and functional indicators of damage that increase in the immediate hours and days following eccentric exercise. These biochemical indicators include elevated levels of serum creatine kinase, urinary 3MH, the cytokines (IL-1, IL-6, and TNF) (Armstrong et al., 1983: Cannon et al., 1991; Evans et al., 1986; Nosaka & Clarkson, 1996). The functional indicators of muscle damage include decreased muscle strength and increased muscle soreness that persists for several days post-exercise in the affected muscle group. From 5-10 days post-exercise, these biochemical and functional indicators of muscle

damage gradually return to normal (Clarkson et al., 1986; Nosaka et al., 1991; Smith et al., 1994).

There is research supporting a training adaptation to muscle damage in that repeated bouts of the same eccentric exercise result in less microscopic evidence of damage and a blunted response of some of the indicators of muscle damage, particularly serum CK (Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1990). Despite the great volume of literature regarding muscle damage, the mechanism for this adaptation remains to be determined. In addition, researchers evaluating mechanisms, characteristics, or interventions in muscle damage must take into the account this adaptation effect and may want to include several indicators of muscle damage in their project.

Although serum testosterone, cortisol, and growth hormone responses to resistance exercise may vary due to the volume, intensity, or rest periods of the protocol (Kraemer, 1988), there is little research that examines the hormonal responses specifically following eccentric resistance exercise. Influence of these hormones with any treatment interventions following eccentric resistance exercise have yet to be examined. The role of insulin as an anaboolic hormone associated with muscle glycogen and muscle protein synthesis is well characterized (Biolo et al., 1995; Brillon et al., 1995; Roy & Taronopolsky, 1998; Zawadzki et al., 1992). In addition, there is evidence for eccentric exercise to induce a temporary insulin resistance in muscle 48 hr following exercise (Kirwan et al., 1992). This insulin resistance could interfere with the muscle's ability to resynthesize glycogen and synthesize new muscle protein.

Several studies that examined nutritional interventions following high-intensity swimming and running have indicated the potential for carbohydrate and carbohydrate-protein beverages to lessen serum indicators of muscle damage (Cade et al., 1991) or inflammation (Nieman et al., 1997). Studies which examined nutritional interventions following resistance exercise have reported decreased muscle protein breakdown following carbohydrate supplementation (Roy et al., 1997) or a hormonal environment

where muscle protein synthesis is favorable following post-exercise consumption of a carbohydrate or carbohydrate-protein supplement (Chandler et al., 1994). Elevated serum insulin in the post-exercise period may mediate these effects (Roy & Tarnopolsky, 1998; Roy et al., 1997). The effects of a carbohydrate or carbohydrate-protein supplement consumed following eccentric resistance exercise to potentially lessen indicators of muscle damage, protein breakdown, and inflammation have yet to be examined.

It is possible to induce a moderate amount of glycogen depletion following resistance training exercise (Pascoe et al., 1993; Roy & Tarnopolsky, 1998; Tesch et al., 1986). Furthermore, several studies have shown the muscle glycogen resynthesis is impaired from 24-72 hr following high-force eccentric resistance exercise (Costill et al., 1990; Doyle et al., 1993; Widrick et al., 1992). Although it may be possible that high carbohydrate consumption following eccentric resistance exercise may enhance glycogen resynthesis (Costill et al., 1990), this hypothesis has not yet been examined. However, following resistance training exercise that contains an eccentric component, both carbohydrate and carbohydrate-protein supplements consumed immediately and 1 hr post-exercise resulted in similarly elevated muscle glycogen levels 4 hr post-exercise (Roy & Tarnopolsky, 1998). Therefore, the potential exists for post-exercise carbohydrate and carbohydrate-protein supplements to enhance glycogen resynthesis after eccentric resistance exercise.

Chapter III

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Effect of Post-Exercise Macronutrient Intake on Glycogen Resynthesis and Muscle Damage Induced By Eccentric Resistance Exercise

Janet R. Wojcik[1], Janet Walberg Rankin[1], Lucille L. Smith[2]

Running Head: Macronutrient Intake and Eccentric Resistance Exercise

[1]Janet R. Wojcik
Department of Human Nutrition, Foods, and Exercise
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0351
Phone: (540) 231-8747
FAX: (540) 231-5448
email: jwojcik@vt.edu

[2]Department of Health and Exercise Science
Appalachian State University
Boone, NC 28608

Abstract

This study examined effects of carbohydrate (C), carbohydrate-protein (CP), or placebo (P) beverages following eccentric resistance exercise on indicators of muscle damage and protein breakdown, glycogen resynthesis, and inflammation. Untrained males cycled for 40 min plus cycling sprints on d 4 evening. On d 5 morning, they performed 100 eccentric leg flexions at 120% of 1-RM and drank C (n=8, 1.25 g C/kg), CP (n=9, 0.875 g C/kg, 0.375 g protein/kg), or P (n=9) beverages immediate post-exercise (IPE) and 2 hr later. At 3 hr, insulin was higher for CP (24.6 ± 15.5 μIU/ml) and C (17.2 ± 10.9 μIU/ml) than P (5.3 ± 0.4 μIU/ml, P<.05). Glycogen was low IPE, partially recovered at 24 h, normal by 48 h (P<.01) with no differences among groups. Serum creatine kinase increased (P<.01) on d 5 and was highest for C (P<.05). On d 5, urinary 3-methylhistidine was lower for CP versus C (P <.05). Interleukin-6 peaked at 6 h on d 5 (P<.01) with no group effects. Although glycogen was not affected, a CP beverage may be beneficial for reducing muscle damage, protein breakdown, and inflammation on the day of eccentric resistance exercise.

Keywords: creatine kinase, 3-methylhistidine, protein, insulin, cytokines

Eccentric muscle contractions, production of muscular force while a muscle lengthens, have been demonstrated to produce structural damage to the muscle and induce protein breakdown. Although mechanical stress to the muscle during eccentric contraction may initiate muscle damage, a secondary delayed metabolic response of inflammatory and degenerative changes may follow the mechanical event (16). This damage to the muscle from eccentric exercise is associated with muscle soreness, which typically develops in 24-48 h following novel eccentric exercise. In addition, muscular function and performance are reduced in the days following eccentric exercise (13, 14, 29, 30, 31).

Several biochemical indicators associated with muscle damage are released from the muscle in response to injury. The enzyme creatine kinase (CK) has been reported to increase in the bloodstream within the first 24 h following high-force eccentric resistance exercise and peak approximately 72 h post-exercise (7, 8, 10, 13, 27, 28, 30). Increased urinary levels of an amino acid metabolite from actin and myosin breakdown in muscle, 3-methylhistidine, have been reported following intense resistance exercise which also contains an eccentric component (20, 33, 34).

Furthermore, increased serum levels of the cytokines Interleukin-1 (IL-1), Interleukin-6 (IL-6), and tumor necrosis factor-α (TNF) have been detected following eccentric exercise as part of the inflammatory response (4,18). These cytokines are associated with increased muscle proteolysis (15,16), which may add to the body's free amino acid pool for enhanced hepatic protein synthesis. Furthermore, it has been reported that untrained persons may show a more robust IL-1 response in the immediate hours following eccentric exercise (18).

Several research groups have found impairment in glycogen resynthesis up to 72 h in muscle that underwent eccentric resistance exercise (11, 12, 41). The mechanism for this finding remains to be clarified. One potential explanation for this phenomenon is an increased competition for available glucose between inflammatory cells and muscle cells in the post-exercise period (11).

Although it may not be possible to attenuate damage caused by mechanical stress to the muscle, it may be possible to influence the delayed metabolic responses by treatment interventions. A limited amount of research has examined nutritional interventions to attenuate muscle damage or to provide an environment favorable for replenishing muscle glycogen and protein. For example, Cade et al. (3) found that a milk-protein supplement consumed after swimming exercise was superior to a carbohydrate-electrolyte drink or water with respect to diminished serum CK response. It was hypothesized that the milk-protein supplement provided amino acids needed to resynthesize catabolized proteins and, thus, allowed for a more rapid recovery. Although their findings were provocative, interpretation is somewhat difficult because the milk-protein supplement varied in volume, and non-randomization of some treatment interventions could not rule out an order effect.

Chandler et al. (6) showed carbohydrate and carbohydrate-protein beverages consumed following resistance exercise produced higher serum insulin for up to 8 h following exercise when compared to a protein beverage, or water. Higher serum growth hormone was seen in the carbohydrate-protein group at 6 h following exercise. These results were interpreted as a favorable environment for muscle protein and glycogen synthesis. Although this study utilized a resistance training protocol, it did not emphasize the eccentric portion of the muscular work or examine indicators of muscle damage or performance.

Accordingly, it is possible that the type of nutritional supplement consumed after eccentric resistance exercise may influence muscle protein proteolysis secondary to the diminished inflammatory response as well as promote a hormonal environment favorable for glycogen and protein synthesis. Enhanced recovery from eccentric exercise could translate into increased muscular performance and/or decreased muscle soreness. Therefore, the purpose of this study was to examine whether a carbohydrate (C), carbohydrate-protein (CP), or placebo (P) beverage consumed following eccentric resistance exercise influenced markers of skeletal muscle damage and catabolism (serum CK and cortisol, urinary 3MH), inflammation (IL-1, IL-6, TNF), muscle glycogen

resynthesis, markers of muscle protein synthesis (serum insulin and testosterone), and muscular performance and soreness.

METHODS

Subjects. Twenty-seven healthy but untrained male subjects volunteered for the study, which was approved by the Institutional Review Board at Virginia Polytechnic Institute and State University. One subject was asked to withdraw from the study because of syncope during a blood draw. Subjects were recruited based on the following criteria: (a) Untrained fitness status with particular emphasis on no resistance training involving the legs or regular running for at least 6 months, (b) no medical contraindications such as chronic disease or orthopedic limitations, (c) no lactose intolerance or milk allergy, (d) no allergy to local anesthetics, (e) no apparent difficulties during blood draws, and (f) availability during the proposed data collection periods. Average activity level for the subjects was 134 ± 25.9 min per week. While 6 subjects were sedentary, 10 participated in recreational activities such as hiking, racquet sports, softball, and basketball, 4 subjects performed resistance training using their upper body only, and 6 subjects performed a combination of recreational activities and upper body resistance training.

Approximately one week prior to the experimental period, subjects reported to the laboratory for testing. Body composition was determined using skinfold measurements from the chest, abdomen, and thigh (23). Peak oxygen uptake (VO_2) was measured using indirect calorimetry (CPX/D, Medical Graphics Corp., St. Paul, MN) and an incremental protocol on a cycle ergometer (Monarch 818E, Stockholm, Sweden). The initial resistance on the cycle flywheel was 0.5 kg, and the resistance was increased by 0.5 kg following each 2 min stage. Heart rate and rhythm were continuously monitored by telemetry, and heart rate was recorded every minute. Ratings of Perceived Exertion (RPE) (1) were obtained at the end of every stage and at the time of test termination. The test was terminated when the subject could no longer maintain the 60 rpm pedaling pace despite encouragement. Peak VO_2 was calculated as the average VO_2 over the final minute of exercise.

Dominant leg isotonic one-repetition maximum (1-RM) was measured on an isotonic leg extension station (Nautilus, Independence, VA). Each subject's 1-RM was determined by increasing or decreasing the resistance on the weight stack until the subject could only perform one repetition through full range of motion at a given weight. A 2.27 kg adapter was added to the weight stack to enhance precision. One minute of rest was given between trials. Quadriceps peak torque, work performed with the maximal repetition, and total work for 5 maximal-effort repetitions were measured on the dominant leg at a test speed of 60^{o}/s using a Biodex isokinetic dynamometer (Biodex Corp., Shirley, NY). A one-way analysis of variance revealed no group differences in baseline subject characteristics. Thus, the pooled subject characteristics are shown in Table 1.

Experimental Design. The experimental period lasted 9 days plus the morning of d 10. An outline of the study design is shown in Figure 1. Subjects consumed a controlled diet for 9 days, and the first 4 days were considered a baseline period where body weight was measured and energy intake was adjusted in order to maintain body weight. Twenty-four hour urine collections were completed on d 3-9. Fasted blood samples were obtained from the antecubital vein on the mornings of d 4-10.

On the evening of d 4, subjects reported to the laboratory to perform 40 min of stationary cycling at 70% of peak VO_2. Heart rate and rhythm were continuously monitored by telemetry, and heart rate and RPE were recorded at 5 min intervals. A rest period of up to 2 min was allowed if subjects became fatigued during the cycling. This endurance exercise was followed by 5 bouts of 1-min maximal cycling sprints interspersed with 2 min of light pedaling. Following the exercise bout, the subjects were given a low-carbohydrate snack (290 kcal, 10% carbohydrate, 21% protein, 69% fat). The rationale for this snack was to keep the subjects' glycogen levels low until they performed the eccentric resistance exercise the following morning.

On the morning of d 5, subjects reported to the laboratory to perform 10 sets of 10 repetitions of isotonic eccentric quadriceps contractions with their dominant leg using the same leg extension machine on which the 1-RM was previously determined. Resistance

was set at 120% of 1-RM. Research assistants raised the weight stack while the subject was asked to lower the weight for a 4 s count. Maintenance of this count became more difficult for the subjects as the sets progressed. One minute rest was given between each set. Additional blood samples were taken immediately (IPE), 3 h (3 h PE) and 6 h (6 h PE) post exercise.

Immediately after the eccentric resistance exercise and blood sampling, subjects underwent a needle muscle biopsy from the vastus lateralis of the exercised leg using the suction technique (17). Additional muscle biopsies were taken 24 h (24 h PE) and 72 h (72 h PE) post eccentric exercise. A new incision was made approximately 3 cm away from the previous incision for subsequent biopsy samples (10). The biopsies were quickly weighed, frozen in liquid nitrogen, and stored at -80^{o}C until analysis. Muscle glycogen was assessed spectrophotometrically using the method of Lo et al. (26).

Following the muscle biopsy procedure and 2 h later, subjects were randomly assigned to receive either C (1.25 g C/kg, Gatorade, Chicago, IL), CP (0.875 g C/kg, 0.375 g protein/kg, skim milk with strawberry-flavored Nestle Quik, (Nestle Corp., San Francisco, CA), or aspartame-flavored placebo (P) (Crystal Light, Kraft Foods, Inc., White Plains, NY). Both C and CP were made as 13% solutions.

Quadriceps muscular soreness was assessed on d 5 prior to, IPE, 3 h, and 6 h post eccentric exercise as well as the mornings of d 6-10. This assessment was conducted while the subjects were resting following the blood draws. Subjects were asked to rate their overall quadriceps soreness on an ordinal 1-10 scale (1 = No Soreness, 10 = Unbearable Soreness; 6). In addition, quadriceps isokinetic exercise performance was assessed 24, 48, and 72 h post eccentric resistance exercise.

Dietary Control. The controlled diet consisted of a meat-free, 3-d rotating menu (21) with a macronutrient content of 55% carbohydrate, 30% fat, and 15% protein. Initial energy was set at 3,000 kcal/d. Subjects who experienced small weight losses and/or expressed hunger were supplemented with Ensure (Abbott Laboratories, Columbus, OH) since it provided additional energy while maintaining the macronutrient breakdown of the

diet. Since the subjects were not housed overnight on campus, they ate all of their breakfasts and dinners in a foods teaching laboratory and returned home each night. For lunches and snacks, the subjects received a small insulated cooler packed with their pre-measured food items. Subjects were instructed to consume no other foods during the experimental period. All foods were weighed and measured prior to serving and again if there was any waste. Diet record check-off sheets were turned in on a daily basis, and any Ensure consumed or food not eaten was accounted for.

On the day of the eccentric resistance exercise (d 5), diets were adjusted in kcal and macronutient content for the C and CP groups to account for the experimental beverages so they could be equivalent to the kcal and macronutrients provided to P. Dietary menus were constructed and subject check-off records were analyzed using Nutritionist IV software (First Data Bank, San Bruno, CA).

Urine Samples. Subjects completed 24 h urine collections for experimental d 3-9. Samples were collected in polypropylene bottles with 1 ml 50% hydrochloric acid as a preservative. Collections began with the second void of the day and were turned in each morning. Total volume was recorded, and aliquots were frozen at -20^{o}C. Urinary 3MH was measured by an amino acid analyzer (PICO.TAG, Waters Association, Milford, MA). The interassay coefficient of variation (CV) was 3.1%. Urinary creatinine was analyzed spectrophotometrically by manual assay procedure using a commercially available kit (Kit #555, Sigma Chemical Co., St. Louis, MO). All samples for a subject were analyzed in duplicate during the same assay.

Blood. Blood samples (10 ml) were drawn from the antecubital vein and placed into heparinized tubes. All samples were placed in an ice bath for 30 min to clot and were centrifuged at 3,000 rpm for 15 min at 4^{o}C. Aliquots of serum were stored at -20^{o}C until analysis. All samples for a subject were analyzed in duplicate during the same assay.

Serum glucose and serum CK were analyzed spectrophotometrically using manual assay procedures from commercially available kits (Kits #520 and #47, respectively, Sigma Chemical Co., St. Louis, MO). Serum insulin, growth hormone

(GH), testosterone, and cortisol were analyzed by radioimmunoassay using commercially available kits (Diagnostic Products Corp., Los Angeles, CA). The intra-assay CV for GH was 17.9% while the interassay CV was 13.9%. All other intra-assay and interassay CV's for the hormones were less than 5%. Serum IL-1, IL-6, and TNF were analyzed by high-sensitivity enzyme immunoassay using commercially available kits (Quantikine HS, R & D Systems, Minneapolis, MN).

Statistics. Data were analyzed using a repeated measures analysis of variance (ANOVA) to assess group by time interactions (Statistical Analysis Systems [SAS], Cary, NC). The values for morning serum CK underwent a logarithmic transformation to normalize the data. A repeated measures analysis of covariance was used for body weight using body weight on experimental d 1 as the covariate. One way ANOVA was used to examine 3MH excretion on d 5, energy intake, and macronutrient data. Student Newman-Keuls post-hoc analyses were employed in the presence of significant F-ratios. When the post-hoc analyses from repeated measures ANOVA on urinary 3MH revealed significant differences between groups on day 5, a separate one-way ANOVA was performed for 3MH on this day only. Statistical significance was set *a priori* at $P < 0.05$. Data are reported as means ± SE.

RESULTS

Body Weight and Energy Intake. Subjects maintained stable body weight throughout the study (Fig. 2). Energy intake per day averaged 3048 ± 232 kcal for CP, 2972 ± 237 kcal for C, and 3090 ± 439 for P and was not statistically different between groups. There was a statistically significant ($P < 0.05$) but minimal difference between percent energy from carbohydrate between C (56.2% ± 0.5) and P (55.5% ± 0.5), but both of these were not different from CP (55.7% ± 0.2). It is unlikely this difference in percent energy carbodhydrate between C and P is of physiological relevance.

Indicators of Muscle Damage. On d 5, lowest 3MH was seen for CP versus C ($P < 0.05$). (Table 2) Similar patterns ($P < 0.05$) were seen for d 5 3MH relative to body weight, and

lean body mass. No other changes were seen in 3MH over the experimental period (Table 2). Serum CK increased for C, CP, and P IPE, 3 h, and 6 h post-exercise ($P < 0.01$). Serum CK IPE, 3 h and 6 h post-exercise was higher for C vs. CP and P ($P < 0.05$)(Fig. 3). The lowest increase in CK on day 5 was seen for CP (NS). Fasted serum CK increased over the mornings of d 4-10 ($P < 0.01$) with peak CK on d 7 (Table 3). The treatment beverages had no effect on fasted serum CK in the days following the eccentric resistance exercise.

Quadriceps isokinetic peak torque was depressed 24% from baseline on d 6 and remained depressed 21% on d 8 ($P < 0.01$; Fig. 4). There was no interaction effect with C, CP, or P. Work done with the maximal repetition and total work over the 5 repetitions followed the same pattern. Muscle soreness peaked at 3.4 ± 0.5 for C, 4.3 ± 0.8 for CP, and 4.1 ± 0.5 for P on a 1-10 scale (7) on d 7 and fell to baseline by d 9. Soreness was not influenced by treatment beverage. (Fig. 5). Soreness also significantly increased over d 5 ($P < 0.01$) and was also not influenced by C, CP, or P (Table 4).

Skeletal Muscle Glycogen and Indicators of Glycogen and Muscle Protein Synthesis. Muscle glycogen was low across all groups on d 5, slightly lower than average values 24 h following eccentric exercise, and at normal resting levels expected for this untrained population by 72 h following eccentric exercise ($P < 0.01$) (Fig. 6). Consumption of the CP, C, and P beverages had no effect on glycogen resynthesis 24 and 72 h following eccentric exercise. At 3 h post-exercise on d 5, serum insulin was higher for CP and C than for P ($P < 0.05$) (Fig. 7). At 3 h post-exercise on d 5, serum glucose was higher for CP (4.7 ± 0.3 mmol/l) and P (4.6 ± 0.1 mmol/l) than for C (4.3 ± 0.2 mmol/l; $P < 0.05$) (Table 4).

Serum testosterone fell 30% over d 5 for all groups ($P < 0.01$). The testosterone at 3 h on d 5 was lower for C and CP versus P ($P < 0.05$) (Fig. 8). Morning serum testosterone showed significant changes over the experimental days ($P < 0.01$) with a 7% drop across all groups from d 4-5 (Table 3). Morning serum testosterone was not affected by treatment beverage. Most serum growth hormone was below detectable levels

of the assay (1.4 ng/ml). Growth hormone increased 200% after eccentric exercise on d 5, but with no interactions with C, CP, or P (Table 4).

Indicators of Inflammation. No changes were seen in serum IL-1 either on d 5 or over the mornings of d 4-10 (Table 3, Table 4). On d 5, IL-6 rose significantly over time ($P < 0.01$), but the increase was smallest for the CP group (NS) (Fig. 9). The values for IL-6 on the mornings of d 4-10 showed no changes (Table 3). Serum TNF fell over d 5 ($P < 0.01$) but showed no interaction with C, CP, or P (Table 4). Significant changes in serum TNF were seen over the morning values on d 4-10 ($P < 0.01$) with highest values on d 5 and no interaction with treatment beverage (Table 3). Serum cortisol fell 50% over d 5 ($P < 0.01$) (Table 4). Morning serum cortisol fell 24% over d 6-8 and rose to baseline d 9-10 ($P < 0.01$) (Table 3). C, CP, and P had no effects on either the d 5 or morning serum cortisol responses.

DISCUSSION

This study suggests that consumption of a carbohydrate or carbohydrate-protein beverage following eccentric resistance exercise has potential to facilitate a hormonal environment favorable for muscle glycogen and muscle protein synthesis. Consumption of a carbohydrate alone or in combination with protein stimulates the β cells of the pancreas to release insulin which facilitates entry of glucose and amino acids into the muscle cells (32, 39). The serum insulin responses in our study for both CP and C at 3 h post-exercise were similar to each other but different from P. These responses at 3 h post-exercise support those seen by Chandler et al. (6) who examined hormonal responses after consumption of carbohydrate-protein, carbohydrate, and protein supplements or water following a resistance circuit training exercise. In their study, the carbohydrate-protein and carbohydrate beverages consumed immediately and 2 h post-exercise produced significantly higher serum insulin levels compared to protein and placebo at 1 h post-exercise and significantly higher responses than placebo at 3 h post-exercise. Thus, higher serum insulin observed for CP and C in the post-exercise period for both studies can be interpreted as favorable hormonal environment for muscle growth.

At 24 h post-eccentric exercise, skeletal muscle glycogen levels were somewhat lower than normal resting values across all treatments, which is consistent with other studies (11). However, there was no benefit of consuming either CP or C beverage relative to the P beverage on muscle glycogen 24 hr following eccentric resistance exercise. The higher serum insulin levels for CP and C compared to P at 3 h could have influenced the rate of skeletal muscle glycogen resynthesis in the more immediate hours following exercise as shown in a study by Roy and Tarnopolsky (35). These researchers examined muscle glycogen resynthesis for carbohydrate/protein/fat, carbohydrate, and placebo beverages consumed immediately and 1 h following a resistance circuit exercise session. Both their treatment beverages produced comparably elevated serum insulin levels 2.5 h post-exercise that were significantly different from the placebo. As a result, the rate of skeletal muscle glycogen resynthesis taken from biopsies at 4 h post-exercise was significantly higher for the treatment beverages versus the placebo and control muscle from the non-exercised contralateral leg (35). Thus, future studies examining the effect of post-exercise carbohydrate-protein beverages should take muscle biopsies earlier than the 24 h time point in our study.

The serum CK response in our subjects was highly variable, consistent with previous studies (8, 11, 28, 29). On experimental d 8, 3 d following the eccentric resistance exercise, 12 subjects had serum CK levels less than 100 U/L, 12 subjects had serum CK ranging from 120-1,700 U/L, while 2 subjects had peak serum CK at approximately 10,000 U/L. In addition, 8 subjects demonstrated increased serum CK prior to eccentric exercise on d 5 in comparison to the morning of d 4. This suggests the high-intensity cycling exercise the night before the eccentric resistance exercise initiated some muscle damage in these untrained subjects. An analysis of covariance with serum CK on d 5 morning as the covariate did not alter the statistical results. However, the issue remains that the cycling exercise may have already initiated a serum CK and inflammatory response in these particular subjects. Therefore, the time course of CK response from the eccentric resistance exercise in these subjects may have been altered in these subjects relative to studies that did not include intense aerobic exercise combined with eccentric resistance exercise. Studies that examine eccentric aerobic exercise such

as downhill running often report peak serum CK values at 24 h post-exercise (2). On the other hand, studies which examine high force eccentric exercise utilizing resistance training report peak serum CK values 3-5 d post-exercise (8, 13, 30, 31). Furthermore, the peak serum CK responses are often less in downhill running when compared to high force eccentric exercise (2).

Although many studies involving novel eccentric exercise have found a several-hundred-fold increase in serum CK response (11, 14, 29), there have been reports of eccentric exercise eliciting only modest increases in serum CK responses. In contrast to the study by Costill et al. (11) where aerobic cycling followed eccentric resistance exercise, a subsequent study from the same laboratory (41) did not find a significant increase in CK response when eccentric resistance exercise followed aerobic cycling exercise. Since pre-exercise CK values in this study (41) were somewhat elevated (227 ± 75 U/L) over normal values of less than 100 U/L (Sigma Chemical Co., St. Louis, MO), perhaps some muscle damage was already initiated in these subjects which lead to the modest CK response following the eccentric resistance exercise. Using a similar protocol where eccentric resistance exercise followed aerobic cycling and cycling sprints, Doyle et al. (12) reported significant increases in CK responses from their baseline values. However, their peak CK values 72 h post-exercise were only moderately elevated to 444 ± 157 U/L from higher-than-normal pre-exercise values of 147 ± 23 U/L.

The subject characteristics in the above studies may have influenced the CK response. The study which found the greatest increase in serum CK using a protocol which combined eccentric resistance exercise with cycling exercise (11) utilized subjects who were not involved in any eccentric activity, while other studies that utilized subjects who were endurance trained but not performing resistance exercise involving their leg musculature found smaller increases in serum CK (12, 41). Several studies have shown that prior eccentric exercise can blunt the effect on the serum CK response to subsequent eccentric exercise. This adaptation develops rapidly in the days following exercise--even before the serum CK returns to normal levels--and can last for up to six months (8, 30). The mechanism for this rapid adaptation remains unclear but may include both cellular

and neural adaptations (9). In addition, the intensity of the eccentric exercise required to elicit this protective response need not be very high (9, 13). Thus, the recreational activities performed by subjects in our study may have contributed to the variability in the CK response.

Along with the increased serum CK response, eccentric resistance exercise is associated with the development of significant muscle soreness which typically develops within 24 hours and peaks about 72 hours post-exercise (11, 12, 31, 38). We were able to elicit significant muscle soreness that increased over d 5 and peaked approximately 3-4 days post eccentric resistance exercise, but this soreness was only of a moderate intensity. Like serum CK, muscle soreness was also variable in our study. Subjects reported ratings 3-4 d post-exercise that ranged from 1 (no soreness) to 8 (greater than very strong soreness) on a 1-10 scale (7). Moreover, we were unable to influence muscle soreness at any of these time points as a result of beverage consumption post-eccentric resistance exercise.

About a 50% loss of muscular force production is typically seen immediately following eccentric resistance exercise (14, 29, 30, 31). This decreased muscle function persists for several days and gradually returns to normal by 10-12 d post-exercise (9, 29). Our eccentric resistance protocol evoked significant decreases in isokinetic peak torque (Figure 4), work done with the maximal repetition, and total work for five repetitions at 24 h post-exercise which slowly began recovery at 48 and 72 h post-exercise. Post-exercise beverage consumption did not affect muscle function from 24-72 h following the exercise. Although researchers have attempted to link decreased muscle function to muscle soreness, the muscular deficits persist after muscle soreness disappears by 5-7 d following exercise (9, 29).

There have been conflicting results regarding acute or chronic resistance exercise training on increased 3MH excretion as an indicator of muscle breakdown. One study, following initiation of a resistance circuit exercise, found increased 3MH excretion by 3 d that persisted throughout the length of the 10 d study (34). Another study that examined 3MH excretion over a 28 d resistance training program found gradual increases in 3MH

over this time period (20). We did not see any changes in 3MH on experimental days 6-9. It is possible that if we extended urine collection we may have seen increases in 3MH at time points beyond four days post-exercise. The studies that reported increases in 3MH used whole body circuit resistance exercise and multiple workout sessions during their experimental periods (20, 34). We used the quadriceps muscle group for only one exercise session. Studies which used only one or two resistance training sessions did not report changes in 3MH (21, 22), while another study using one resistance exercise session actually found decreased 3MH excretion 24 h following exercise (33).

An interesting finding from our study was the lower 3MH excretion seen for the carbohydrate-protein supplement on the day of eccentric exercise. This finding supports a recent study (36) which examined the effect of 1 g/kg carbohydrate consumption immediately and 1 h after 80 unilateral leg extension exercise at 85% of 1-RM. They found that urinary 3MH excretion in the 24 h following exercise was significantly lower for the supplement group versus a placebo group. The higher serum insulin observed for the supplement group may have played a role in the reduced muscle protein degradation by 3MH (35).

We found lower 3MH excretion for CP than C on d 5 despite comparable serum insulin levels for these groups 3 and 6 h post-exercise on the same day. Thus, a characteristic of the carbohydrate-protein beverage other than its influence on serum insulin appeared to affect muscle protein breakdown. Nonetheless, it is an interesting possibility that a nutritional supplement consumed following novel eccentric resistance exercise may lessen muscle damage following the exercise.

Increases in serum testosterone have been associated with muscle protein synthesis (5, 25), whereas increases in serum cortisol have been associated with both stimulating muscle protein breakdown and inhibiting protein synthesis (5, 37). Other experimental protocols elicited increases in serum testosterone (24, 40) and serum cortisol (24, 27, 28) following heavy resistance exercise, but these responses may be related to a threshold intensity of exercise, volume of the training program, duration of rest period between sets, or amount of muscle mass exercised (25). Our eccentric exercise protocol may not

have been of sufficient intensity, volume, or muscle mass to elicit increased serum testosterone or serum cortisol immediately post-exercise in our subjects. Furthermore, any changes in serum cortisol and morning serum testosterone in our subjects were well within normal physiological limits (19). Post-exercise nutrient consumption did not influence either serum cortisol at any time point or morning serum testosterone.

At 3 h post-exercise on d 5, lower serum testosterone was observed for those subjects who consumed either nutrient-containing beverage compared to the placebo. These results corroborate those obtained by Chandler et al. (6) who found lower serum testosterone from 2-5 h post resistance circuit exercise for carbohydrate, protein, and carbohydrate-protein supplements versus placebo. Measurement of luteizining hormone during their carbohydrate trial showed no changes during these same time points. Therefore, they hypothesized that increased clearance of serum testosterone instead of reduced secretion may be responsible for the lower serum testosterone observed following consumption of their supplements. A recent study (40) which examined 17 days of diet records from males who performed 5 sets of 10-RM bench pressing and 30% 1-RM jump squats reported significant negative correlations between serum testosterone and dietary protein ($r = -0.71$) and protein/carbohydrate ratio ($r = -0.59$). They hypothesized macronutrient-based alterations in either metabolism of testosterone or production of sex hormone binding globulin influenced these relationships. Yet, it is interesting to note that manipulation of macronutrients following resistance training exercise may influence the serum testosterone response. The implications of altered post-exercise serum testosterone responses on muscle protein synthesis remain to be clarified for persons undergoing resistance training.

In summary, carbohydrate-protein and carbohydrate supplements consumed immediately and 2 h following novel eccentric resistance exercise can promote a hormonal environment favorable for muscle glycogen and contractile protein synthesis. This environment may be stimulated by the comparably elevated serum insulin levels observed for these beverages during the immediate hours post-exercise. Further benefits of the carbohydrate-protein beverage include lessening of the delayed metabolic response

to muscle damage and inflammation as seen by lowest changes in serum CK and IL-6. A carbohydrate-protein supplement may be beneficial for reducing muscle protein breakdown as measured by urinary 3-methylhistidine on the day of eccentric resistance exercise, an effect which may also be mediated through its effect on serum insulin levels. Therefore, resistance trainers may choose to consume carbohydrate-protein supplements following an acute bout of intense or novel eccentric resistance exercise.

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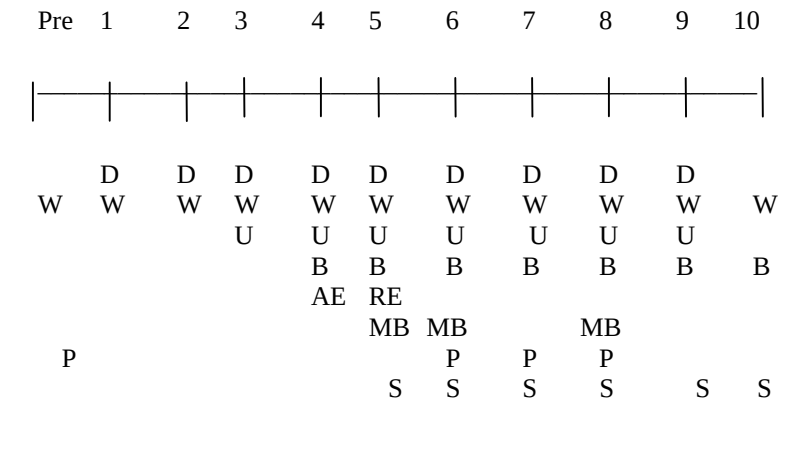
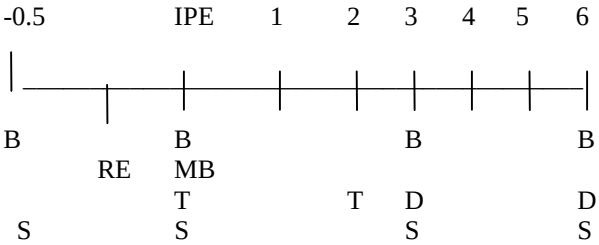
Figure 1. Study design.





D - Controlled diet
U - 24-h urine collection
B - Blood draw
AE - Aerobic cycling: 40 min at 70% $VO_{2peak,}$ 5 1-min sprints
RE - 10 sets of 10 eccentric leg flexions (120% 1-RM)
P - Isokinetic performance test
MB - Muscle biopsy
W - Body weight
S - Muscle soreness assessment (1-10 scale)
T - Treatment (carbohydrate, carbohydrate-protein, or placebo)

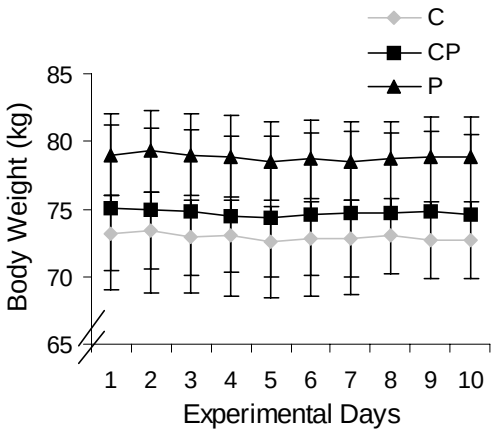


Fig. 2. Body weight during all experimental days.

C, carbohydrate; CP, carbohydrate-protein; P, placebo.

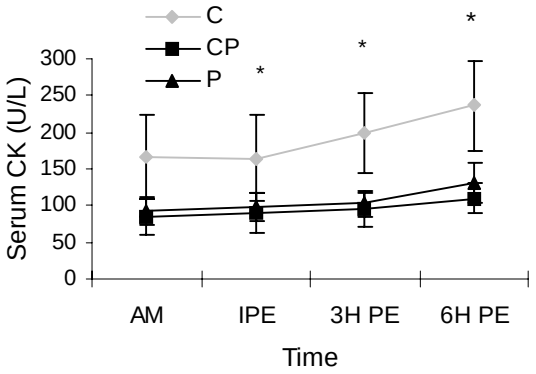


FIG. 3. Serum creatine kinase (CK) prior to and following eccentric resistance exercise. C, carbohydrate; CP, carbohydrate-protein; P, placebo. AM, morning; IPE, Immediate post-exercise; 3H PE, 3 h post-exercise; 6H PE, 6 h post-exercise. $P < 0.01$ for time.

*$P < 0.05$ for C vs. CP and P.

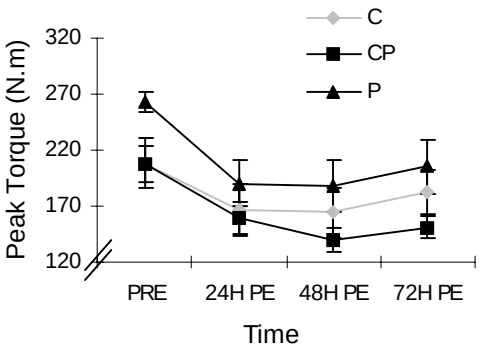


FIG. 4. Isokinetic peak torque measured at 60^{o}/sec.

C, carbohydrate; CP, carbohydrate-protein; P, placebo.

PRE, pre-experimental period; 24H PE,

24 h post-exercise; 48H PE, 48 h post-exercise;

72H PE, 72 h post-exercise. $P < 0.01$ for time.

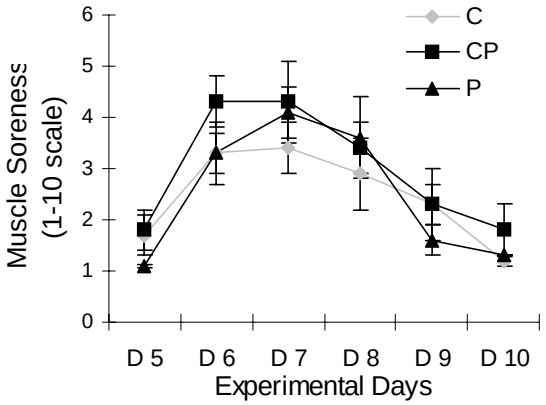


FIG. 5. Morning muscle soreness prior to and following eccentric resistance exercise. C, carbohydrate; CP, carbohydrate-protein; P, placebo. $P < 0.01$ for time.

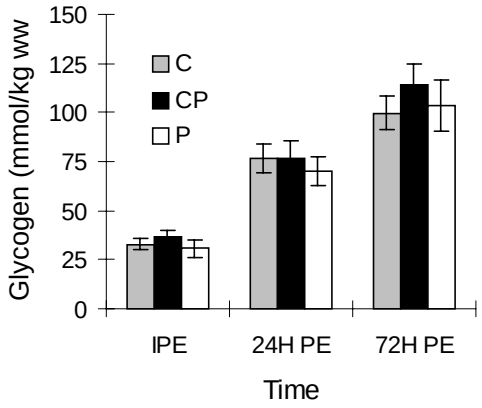


FIG. 6. Skeletal muscle glycogen resynthesis following glycogen depletion and eccentric resistance exercise. C, carbohydrate; CP, carbohydrate-protein; P, placebo. IPE, immediate post-eccentric exercise; 24H PE, 24 h post-exercise;72H PE, 72 h post-exercise. $P < 0.01$ for time.

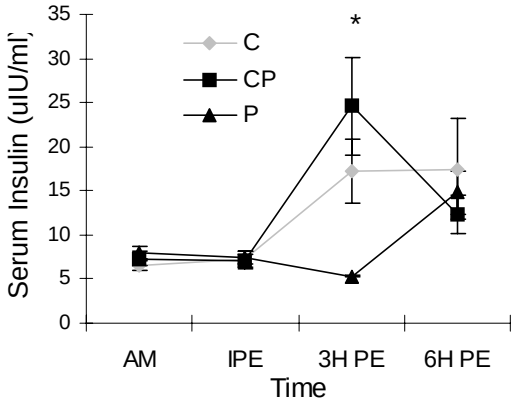


Figure 7. Serum insulin on experimental day 5.

C, carbohydrate; CP, carbohydrate-protein; P, placebo.

AM, morning; IPE, Immediate post-exercise; 3H PE,

3 h post-exercise; 6H PE, 6 h post-exercise.

$P < 0.01$ for time. *$P < 0.05$ for C and CP vs. P.

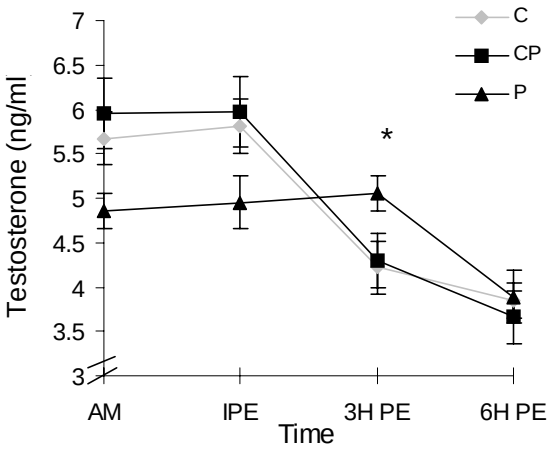


FIG. 8. Serum testosterone prior to and following eccentric resistance exercise. C, carbohydrate; CP, carbohydrate-protein; P, placebo. AM, morning; IPE, Immediate post-exercise; 3H PE, 3 h post-exercise; 6H PE, 6 h post-exercise. $P < 0.01$ for time. *$P < 0.05$ for P vs. C and CP at 3H PE.

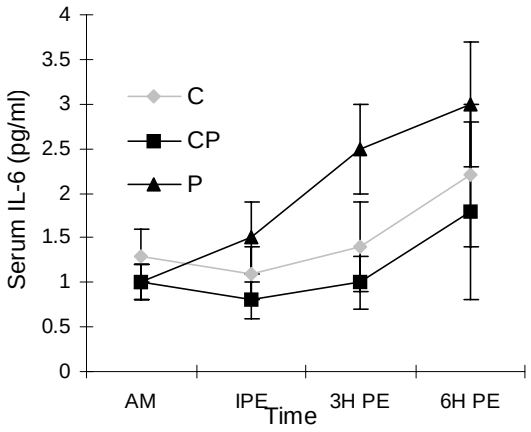


FIG. 9. Serum Interleukin-6 prior to and following eccentric resistance exercise. C, carbohydrate; CP, carbohydrate-protein; P, placebo. AM, morning; IPE, immediate post-exercise; 3H PE, 3 h post-exercise; 6H PE, 6 h post-exercise. $P < 0.01$ for time.

Table 1. Subject characteristics.

Age (yr)	23.5 ± 0.7
Height (cm)	176.3 ± 1.4
Weight (kg)	75.6 ± 2.3
Body Fat (%)	11.7 ± 1.2
VO_{2peak} (ml/kg/min)	41.1 ± 1.0
Dominant Leg 1-RM (kg)	63.9 ± 2.7
Dominant Leg Peak Torque (N·m)	226.8 ± 10.4

Data presented as means ± SE of 26 subjects. VO_{2peak}, peak oxygen uptake; 1-RM, 1 repetition maximum.

Table 2. Urinary 3-methylhistidine (3MH) excretion on (μmol/d) experimental days 3-9 and excretions expressed by body weight (kg), lean body mass (kg lbm) and per g creatinine.

Group	D 3	D 4	D 5	D 6	D 7	D 8	D 9
3-Methylhistidine (μmol/d)							
C	237.3 ± 49.8	200.0 ± 29.0	251.1 ± 22.5[ab]	218.6 ± 9.8	196.6 ± 10.2	211.1 ± 10.0	202.4 ± 15.9
CP	196.2 ± 29.0	175.3 ± 23.2	193.0 ± 13.8[b]	212.8 ± 17.0	201.2 ± 7.0	183.3 ± 24.0	189.9 ± 74.0
P	189.2 ± 22.0	213.5 ± 10.0	235.2 ± 6.8[a]	199.5 ± 18.6	207.7 ± 7.0	221.6 ± 31.7	209.6 ± 13.8
3-Methylhistidine (μmol/kg/d)							
C	3.2 ± 0.5	2.8 ± 0.1	3.5 ± 0.3[ab]	3.0 ± 0.1	2.7 ± 0.2	2.9 ± 0.2	2.8 ± 0.2
CP	2.6 ± 0.2	2.3 ± 0.2	2.7 ± 0.2[b]	2.9 ± 0.1	2.7 ± 0.2	2.5 ± 0.3	2.5 ± 0.2
P	2.5 ± 0.3	2.7 ± 0.2	3.0 ± 0.1[a]	2.6 ± 0.3	2.7 ± 0.1	2.9 ± 0.5	2.7 ± 0.2
3-Methylhistidine (μmol/kg lbm/d)							
C	3.6 ± 0.6	3.1 ± 0.1	3.9 ± 0.4[ab]	3.4 ± 0.2	3.1 ± 0.2	3.3 ± 0.2	3.1 ± 0.2
CP	2.9 ± 0.3	2.6 ± 0.3	3.0 ± 0.2[b]	3.2 ± 0.2	3.0 ± 0.2	2.8 ± 0.3	2.9 ± 0.3
P	2.7 ± 0.3	3.2 ± 0.2	3.5 ± 0.1[a]	3.0 ± 0.3	3.1 ± 0.1	3.3 ± 0.5	3.1 ± 0.2
3-Methylhistidine/Urinary Creatinine (μmol 3MH/g creatinine per d)							
C	171.0 ± 36.4	152.9 ± 25.8	181.1 ± 18.2	143.9 ± 15.9	137.1 ± 12.2	148.2 ± 9.9	149.6 ± 1.2
CP	181.8 ± 31.1	147.6 ± 14.6	184.8 ± 28.5	167.8 ± 25.7	152.9 ± 14.1	165.8 ± 27.8	154.8 ± 6.0
P	114.6 ± 6.3	132.8 ± 6.8	188.1 ± 27.2	123.2 ± 19.4	150.7 ± 15.9	133.5 ± 12.5	145.2 ± 8.4

Data are presented as mean ± SE. C, carbohydrate; CP, carbohydrate-protein; P, placebo. Values with differing superscripts are significantly different ($P < 0.05$).

Table 3. Morning values for serum cortisol, creatine kinase, testosterone, cortisol, and cytokines for experimental days 4-10.

Group	D 4	D 5	D 6	D 7	D 8	D 9	D 10
Cortisol (ng/ml) [a]							
C	165.6 ± 14.8	166.4 ± 42.7	139.5 ± 19.2	145.9 ± 16.7	136.0 ± 13.2	171.1 ± 21.0	172.2 ± 13.0
CP	207.2 ± 8.5	205.6 ± 30.8	152.8 ± 24.2	155.5 ± 19.7	141.3 ± 14.4	178.9 ± 18.7	193.0 ± 20.1
P	187.6 ± 22.1	196.8 ± 17.6	137.5 ± 16.2	155.1 ± 11.9	129.7 ± 12.7	182.9 ± 16.4	198.3 ± 20.0
Creatine Kinase (U/L) [a]							
C	86.4 ± 25.9	165.4 ± 57.5	188.2 ± 44.9	151.3 ± 44.9	400.7 ± 207.5	367.5 ± 194.2	361.1 ± 201.4
CP	67.9 ± 14.2	84.8 ± 23.9	224.2 ± 137	74.4 ± 9.6	1693.9 ± 1613.8	1661.8 ± 4431	1219.3 ±1131.9
P	47.2 ± 9.6	92.1 ± 19.4	141.9 ± 35.6	141.9 ± 35.6	1228.9 ± 986.0	1273.2 ± 841.7	1089.7 ± 80.3
Testosterone (ng/ml) [a]							
C	6.1 ± 0.4	5.7 ± 0.3	5.8 ± 0.3	6.0 ± 0.5	5.8 ± 0.4	6.0 ± 0.3	6.0 ± 0.3
CP	6.2 ± 0.4	6.0 ± 0.4	5.7 ± 0.4	5.8 ± 0.4	5.4 ± 0.3	5.3 ± 0.2	5.3 ± 0.3
P	5.5 ± 0.4	4.9 ± 0.2	5.1 ± 0.3	5.1 ± 0.3	5.2 ± 0.4	5.2 ± 0.3	5.0 ± 0.3
Interleukin-1 (pg/ml)							
C	0.7 ± 0.2	0.7 ± 0.3	0.8 ± 0.4	0.7 ± 0.4	0.8 ± 0.4	0.9 ± 0.3	0.9 ± 0.4
CP	0.6 ± 0.3	0.7 ± 0.4	0.9 ± 0.4	0.8 ± 0.4	0.8 ± 0.3	0.8 ± 0.3	0.7 ± 0.3
P	1.4 ± 0.6	1.4 ± 0.6	1.0 ± 0.3	1.8 ± 0.6	1.4 ± 0.6	0.8 ± 0.5	0.8 ± 0.4
Interleukin-6 (pg/ml)							
C	0.8 ± 0.2	1.3 ± 0.3	1.5 ± 0.6	0.9 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.2
CP	1.5 ± 0.7	1.0 ± 0.2	0.9 ± 0.2	1.2 ± 0.4	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.2
P	1.5 ± 1.3	1.0 ± 0.2	2.1 ± 0.5	1.8 ± 06	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.4
Tumor Necrosis Factor-α (pg/ml) [a]							
C	2.3 ± 0.4	2.7 ± 0.3	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.3
CP	3.4 ± 0.5	3.8 ± 0.8	3.1 ± 0.6	2.8 ± 0.4	2.9 ± 0.4	3.4 ± 0.4	3.1 ± 0.4
P	3.0 ± 0.5	2.9 ± 0.4	2.6 ± 0.4	2.5 ± 0.4	2.2 ± 0.4	2.4 ± 0.4	2.4 ± 0.4

Data are presented as mean ± SE. Samples were obtained prior to (AM), immediately following eccentric resistance exercise (IPE), 3 h (3HPE), and 6 h post exercise (6HPE). Carbohydrate (C), carbohydrate-protein (CP), and placebo (P) beverages were consumed IPE and 2 h post-exercise. [a]$P < 0.01$ for time.

Table 4. Serum glucose, growth hormone, cortisol, cytokines, and muscle soreness on day 5.

Measure	AM	IPE	3HPE	6HPE
Glucose (mmol/l) [a]				
C	4.7 ± 0.1	4.9 ± 0.1	4.3 ± 0.2 [b]	5.4 ± 0.3
CP	4.7 ± 0.2	4.8 ± 0.3	4.7 ± 0.3	4.9 ± 0.2
P	4.4 ± 0.1	4.6 ± 0.2	4.6 ± 0.1	4.8 ± 0.3
Growth Hormone (ng/ml) [a]				
C	2.8 ± 0.9	5.9 ± 0.9	1.5 ± 0.1	1.4 ± 0.0
CP	1.4 ± 0.02	2.5 ± 0.5	1.4 ± 0.0	2.6 ± 0.8
P	1.5 ± 0.05	6.3 ± 1.8	1.9± 0.2	1.4 ± 0.0
Cortisol (ng/ml) [a]				
C	166.4 ± 14.2	168.2 ± 17.6	93.9 ± 13.6	87.2 ± 12.1
CP	205.6 ± 30.8	206.2 ± 15.1	102.6 ± 22.1	78.3 ± 8.5
P	196.8 ± 17.6	211.9 ± 24.5	101.7 ± 9.6	104.3 ± 19.7
Interleukin-1 (pg/ml)				
C	0.7 ± 0.3	0.8 ± 0.4	0.8 ± 0.4	0.8 ± 0.4
CP	0.7 ± 0.4	0.7 ± 0.4	0.6 ± 0.4	0.9 ± 0.4
P	1.4 ± 0.6	1.3 ± 0.3	1.1 ± 0.5	1.0 ± 0.4
Tumor Necrosis Factor-α (pg/ml) [a]				
C	2.7 ± 0.3	2.1 ± 0.4	1.8 ± 0.3	1.7 ± 0.3
CP	3.8 ± 0.8	3.4 ± 0.5	3.0 ± 0.6	3.0 ± 0.6
P	2.9 ± 0.4	2.3 ± 0.3	2.4 ± 0.2	2.1 ± .0.3
Muscle Soreness (1-10 scale)[a]				
C	1.8 ± 0.4	4.9 ± 0.5	3.1 ± 0.7	3.7 ± 0.6
CP	1.8 ± 0.4	4.0 ± 0.8	3.4 ± 0.5	3.6 ± 0.5
P	1.2 ± 0.1	3.2 ± 0.7	2.9 ± 0.4	2.8 ± 0.4

Data are presented as mean ± SE. Samples were obtained prior to (AM), immediately following eccentric resistance exercise (IPE), 3 h (3HPE), and 6 h post exercise (6HPE). Carbohydrate (C), carbohydrate-protein (CP), and placebo (P) beverages were consumed IPE and 2 h post-exercise.
[a]$P < 0.01$ for time; [b]$P < 0.05$ for C vs. CP and P.

Chapter IV
Summaries, Recommendations for Future Research, Theoretical Issues, and Implications

This chapter contains overall summaries of research results pertaining to the influence of carbohydrate-protein, carbohydrate, or placebo beverages on indicators of muscle damage and muscle glycogen resynthesis following eccentric resistance exercise. A discussion of modifications to the current design follows as well as recommendations for future research. Finally, theoretical implications as well as practical implications are presented.

Summaries

Muscle Damage. One of the purposes of this study was to examine whether a carbohydrate-protein supplement consumed following eccentric resistance exercise could lessen indicators of muscle damage when compared to a carbohydrate or placebo beverage. There is a large body of literature that examines mechanisms of muscle damage as well as indicators of muscle damage from microscopic evidence to biochemical indicators such as serum CK and urinary 3MH to functional indicators such as impaired muscular function and soreness (Armstrong et al., 1983; Clarkson et al., 1986; Evans et al., 1986; Friden et al., 1981; Nosaka & Clarkson, 1996; Nosaka et al., 1991; Pivarnik et al., 1989). On the other hand, little research has been conducted on the potential for nutritional supplements consumed following exercise to influence indicators of muscle damage (Cade et al., 1991; Nieman et al., 1996; Roy et al., 1997), despite some indications this is an area for future research (Evans, 1991).

Therefore, one purpose of this study examined if consumption of a carbohydrate-protein beverage immediately and 2 hr following 100 repetitions of eccentric resistance exercise was superior to a carbohydrate or placebo beverage lessening indicators of muscle damage. Two potential time points for treatment effects were proposed: (a) on the day of the eccentric resistance exercise, experimental day 5; and (b) in the days following the eccentric resistance exercise, experimental days 6-10.

Twenty-seven untrained males were recruited for the study, but one participant fainted prior to the blood draw following the eccentric exercise and had to be dropped from the study. The experimental period consisted of 9 full days plus the morning of day 10. All subjects underwent a controlled feeding for days 1-9. Twenty-four hour urine collections were performed on days 3-9. Fasted blood samples were obtained the mornings of days 4-10. All subjects underwent an quadriceps muscle glycogen depletion exercise bout using a cycle ergometer on the evening of day 4. A low carbohydrate snack followed the exercise to keep muscle glycogen reduced. On the morning of d 5 fasted subjects performed 100 eccentric leg flexions of the quadriceps. Additional blood draws were obtained immediately, 3, and 6 hr following the eccentric exercise. Each subject was given the assigned treatment beverage (CP, n =8; C, n = 9; P, n = 9) following the muscle biopsy and 2 hr later. Quadriceps muscle soreness was assessed with each blood draw on day 5 as well as the mornings of days 6-10. Quadriceps peak isokinetic torque, total work for 5 repetitions, and work done with the maximal repetition were assessed pre-experimentally as well as 24, 48, and 72 hr post-eccentric exercise.

All blood, urine, exercise performance, and muscle soreness data were analyzed using a repeated measures analysis of variance (ANOVA) to assess group by time interactions (Statistical Analysis Systems [SAS], Version 6.12 for Windows, Cary, NC). The data for morning CK underwent a logarithmic transformation to normalize the data. Repeated measures analysis of covariance was performed on body weight with body weight on day 1 as the covariate and on day 5 serum CK with the fasted morning CK value as the covariate. A one-way ANOVA was used to examine daily energy and macronutrient data. Student Newman-Keuls post-hoc analyses were employed in the presence of significant F-ratios. When the post-hoc analyses from repeated measures ANOVA on urinary 3MH revealed significant differences between groups on day 5, a separate one-way ANOVA was performed for 3MH on this day only.

On day 5, lowest 3MH was seen for CP versus C ($p < .05$). (Table 2) Similar patterns ($p < 0.05$) were seen for d 5 3MH relative to body weight, and lean body mass. No other changes were seen in 3MH over the experimental period. Serum CK increased

for C, CP, and P over day 5 ($p < .01$) with interaction between treatment beverages ($p < .04$) (Fig. 3). Serum CK immediate, 3 hr and 6 hr post-exercise was higher for C vs. CP and P ($p < .05$). Fasted serum CK increased over the mornings of days 4-10 ($p < .01$) with peak CK on day 7 (Table 4). The treatment beverages had no effect on fasted serum CK in the days following the eccentric resistance exercise.

Serum cortisol fell 50% over day 5 ($p < .01$) (Table 3). Morning serum cortisol fell 24% to 143.7 ng/ml for all groups days 6-8 and rose to baseline days 9-10 ($p < .01$) (Table 4). Consumption of C, CP, and P had no effect on either the day 5 or morning serum cortisol responses.

Quadriceps isokinetic peak torque was depressed by 24% from baseline to 226.1 ± 18.9 N·m on day 6 and remained depressed by 21% on day 8 ($p < .01$; Fig. 4). There was no interaction effect with C, CP, or P. Work done with the maximal repetition and total work over the 5 repetitions followed the same pattern. Muscle soreness peaked at on day 7 and fell to baseline by day 9. Soreness was not influenced by C, CP, or P. (Fig. 5). Soreness significantly increased over day 5 ($p < .01$) and was not influenced by C, CP, or P (Table 3).

These results would support the hypothesis that a carbohydrate-protein beverage consumed following eccentric resistance exercise results in lower excretion of 3MH in the 24 hr following the exercise. These results were seen when 3MH was expressed on an absolute (μmol/day), relative to body weight (μmol/kg/day), or relative to lean body weight (μmol/lbw/day) basis. Furthermore, the increase in serum CK over day 5 was lower for CP than for C but not different from P. Therefore, the hypothesis that a carbohydrate-protein beverage results in lower serum CK in the hours following eccentric exercise cannot be supported.

Since there were no differences between beverages on serum cortisol and muscle soreness on day 5 and CK, cortisol, muscle soreness, and isokinetic exercise performance

in the days following exercise, these results would not support the hypotheses of a carbohydrate-protein beverage lessening these indicators of damage.

Muscle Glycogen Resynthesis. Another purpose of this study was to examine if consumption of a carbohydrate-protein beverage immediately and 2 hr following 100 repetitions of eccentric resistance exercise was superior to a carbohydrate or placebo beverage in promoting higher muscle glycogen resynthesis as well as promoting a hormonal environment favorable for muscle glycogen and muscle contractile protein synthesis. Previous research has shown that glycogen resynthesis is impaired following eccentric exercise (Costill et al., 1990; Doyle et al., 1993; O'Reilly et al., 1987; Widrick et al., 1992).

Studies that examined macronutrient supplementation following concentric aerobic cycling exercise reported higher glycogen resynthesis for supplements which were a carbohydrate-protein combination (Tarnopolsky et al., 1997; Zawadzki et al., 1992). Furthermore, studies that investigated glycogen resynthesis following resistance exercise reported favorable hormonal environments for glycogen resynthesis with consumption a carbohydrate-protein beverage (Chandler et al., 1994; Roy & Tarnopolsky, 1998) as well as comparable muscle glycogen resynthesis rates 4 hr post-exercise for a carbohydrate-protein beverage and a carbohydrate beverage (Roy & Tarnopolsky, 1998).

Subjects, methods, and statistics were identical to those described in the previous section. The repeated measures analysis of covariance with body weight on experimental day 1 as the covariate revealed subjects maintained stable body weight throughout the study (Fig. 2). Energy intake averaged 3048 ± 232 kcal for CP, 2972 ± 237 kcal for C, and 3090 ± 439 for P and was not statistically different between groups. There was a statistically significant ($p < .05$) difference between percent energy from carbohydrate between C (56.2% ± 0.5) and P (55.5% ± 0.5) but not C (55.7% ± 0.2), but this difference is unlikely to be physiologically relevant.

Muscle glycogen was low across all groups on day 5, slightly lower than average values 24 hr following eccentric exercise, and at normal resting levels expected for this untrained population by 72 hr following eccentric exercise ($p < .01$). (Fig. 6). Consumption of the CP, C, and P beverages had no effect on glycogen resynthesis 24 and 72 hr following eccentric exercise. At 3 hr post-exercise on day 5, serum insulin was higher for CP and C than for P ($p < .05$) (Fig. 7). At 3 hr post-exercise on day 5, serum glucose was higher for CP (4.7 ± 0.3 mmol/l) and P (4.6 ± 0.1 mmol/l) than for C (4.3 ± 0.2 mmol/l; $p < .05$) (Table 3).

Serum testosterone fell 30% over day 5 for all groups ($p < .01$). The testosterone at 3 hr on day 5 was lower for C (4.2 ± 0.3 ng/ml) and CP (4.3 ± 0.3 ng/ml) versus P (5.1 ± 0.2 ng/ml, $p < .05$) (Fig. 8). Morning serum testosterone showed significant changes over the experimental days ($p < .01$) with a 7% drop across all groups from days 4-5 to 5.5 ± 0.3 ng/ml (Table 4). Consumption of C, CP, and P had no effect on morning serum testosterone. Most serum growth hormone was below detectable levels of the assay (1.4 ng/ml). Growth hormone increased 200% after eccentric exercise on day 5, but with no interactions with C, CP, or P (Table 3).

These results would not support the hypothesis that a carbohydrate-protein beverage consumed following eccentric resistance exercise results in higher serum insulin 3 hr following eccentric exercise since insulin was numerically higher than the carbohydrate beverage but only statistically significant from the placebo beverage. The same conclusion can be suggested for serum glucose since the carbohydrate-protein beverage was also numerically higher than placebo but significantly from carbohydrate at 3 hr post-exercise on day 5. Although serum testosterone for the carbohydrate-protein and carbohydrate beverages were significantly different from placebo, the direction was opposite that anticipated. Serum testosterone was actually lower 3 hr post-exercise for the carbohydrate-protein and carbohydrate beverages compared to placebo. Therefore, the hypothesis that a carbohydrate-protein beverage results in higher serum testosterone in the hours post-exercise is not supported.

There were no significant differences between beverages on serum growth hormone on day 5 and skeletal muscle glycogen and serum testosterone in the days following exercise. These results would not support the hypotheses of a carbohydrate-protein beverage promoting muscle glycogen resynthesis as well as promoting serum growth hormone and testosterone as indicators of muscle glycogen and muscle contractile protein synthesis over a carbohydrate and placebo beverage.

Suggested Modifications of Research Design

There are several modifications to the design of this study that could have been improved upon in order to lessen variability in the data or to provide more information about responses observed.

The original design of the study called for the use of the Biodex isokinetic dynamometer (Biodex Corp., Shirley, NY) for the subjects to perform eccentric quadriceps contractions. This was because total work could be measured for the exercise and intensity of effort could be monitored. However, pilot testing revealed only the passive mode of the Biodex could be used for eccentric contractions, and the subject had to resist the downward movement by performing an eccentric contraction of the quadriceps. This effort, too, was found to be variable since some pilot subjects had difficulty performing the eccentric contraction. Furthermore, some subjects could exceed the mechanical force capabilities of the Biodex and cause the machine to stop.

Therefore, it was decided the subjects would perform eccentric contractions on an isotonic leg extension exercise station (Nautilus, Independence, VA). The selection of 10 sets of 10 eccentric quadriceps contractions (leg flexions) at an intensity of 120% 1-RM was based on the protocols of Costill et al., (1990), Doyle et al., (1993), and Widrick et al. (1992). Although during the eccentric exercise, subjects initially could maintain a 4 s count to lower the weight stack, maintenance of this count became difficult as the subjects fatigued. This fatigue also could have altered their effort during the exercise session since persons unfamiliar with resistance training or athletics may also be unfamiliar about providing a consistent maximal effort. Therefore, the intensity of the

eccentric protocol could have varied among the subjects and affected some of the variables, particularly serum CK.

The only way to elicit and monitor maximal effort was through consistent verbal encouragement, whereas, intensity of effort on the Biodex could be monitored through visual display from the computer interfaced with the system. It is possible to interface isotonic resistance training equipment with such an apparatus to use in this type of eccentric protocol (Widrick et al, 1992). Perhaps if a specific amount of work or force application for the 100 eccentric contractions were monitored instead of just the actual number of contractions, a less variable response in serum CK may have been observed. Another way to maintain consistent exercise intensity for each subject could have been to gradually decrease the load on the weight stack by a fixed percentage whenever the subject had difficulty maintaining the 4 s count to lower the weight stack. This type of protocol was used in the study by Rodenburg et al. (1993), and by the end of their 30 min eccentric arm protocol only 30% of the original resistance remained on the weight stack.

Potential subjects were purposely screened to target those with untrained fitness status since these individuals would most likely evoke a robust response on targeted indicators of damage, inflammation, and muscle soreness. Once again, subjects with minimal experience in athletics or resistance exercise could have reduced perception about giving a maximal effort during either the 1-RM testing or throughout the eccentric resistance exercise protocol. Although familiarization trials were given for the Biodex and for the maximal aerobic capacity test, some subjects required more than two trials to give consistent maximal effort on the Biodex.

It may have been prudent to obtain a muscle biopsy sample 3-4 hr post-exercise on day 5 since no changes were seen between treatment beverages for skeletal muscle glycogen 24 hr post-exercise in this study. Although 24 hr post-exercise glycogen levels across all groups were somewhat lower than expected for this untrained population at this time point, any treatment effects of the beverages were likely eradicated by this time. Since total energy for day 5 was not different between the beverage groups, it is likely

that the subjects' comparable muscle glycogen levels observed 24 hr post-exercise were a reflection of total energy on this day.

Serum insulin levels for the subjects who consumed the carbohydrate-protein and carbohydrate beverages were significantly elevated over the placebo beverage at 3 hr post-exercise on experimental day 5. The study by Roy & Tarnopolsky (1998) reported comparably elevated serum insulin areas under the curve for 3 hr post-exercise in subjects who consumed carbohydrate-protein and carbohydrate beverages immediately and 1 hr post-resistance exercise. These areas under the curve were three times higher than for a placebo beverage consumed at the same time points. As a result, skeletal muscle glycogen measured 4 hr post-exercise was significantly higher for the two treatment beverages versus placebo and unexercised control muscle in their study. Therefore, we would also likely observed differences in skeletal muscle glycogen between beverages from 4-6 hr post-exercise on day.

Although significant differences in serum insulin levels for the carbohydrate-protein and carbohydrate beverages compared to placebo at 3 hr post-exercise were observed on day 5, more frequent blood sampling such as every 30 min for about 3 hr post-exercise would have given more information about the serum insulin as well as serum glucose responses to the treatment beverages. This would have allowed comparisons with other studies which performed more frequent blood sampling in the post-exercise period (Chandler et al., 1994; Roy & Tarnopolsky, 1998; Zawadzki et al., 1992). In reality, it was not possible to perform more frequent blood sampling than what occurred in our study. Due to technician and physician availability, muscle biopsies were unable to be performed on a weekend. Therefore, data collection took place during the week when the subjects had to work and/or attend classes. If data were collected on the weekends, subjects could have received a venous catheter, and they could have remained in the laboratory while blood samples were taken every 30 min for 3 hr post-exercise.

Recommendations for Future Research

The potential for post-exercise macronutrient consumption to influence indicators of muscle damage and glycogen resynthesis following exercise remains an interesting area for further research. The immediate post-exercise period up to about 72 hr post-exercise may be a more relevant time period for evaluation since this research and others (Nieman et al., 1997; Roy & Tarnopolsky, 1998; Roy et al., 1997) have reported treatment effects of interventions in this time frame.

It may be interesting to examine post-exercise macronutient consumption on indicators of muscle damage in experienced versus inexperienced weight trainers to evaluate if nutritional interventions are more effective when subjects are trained or untrained. A study which examined indicators of muscle damage in these populations (Paul et al., 1989) reported 40% higher serum CK for the untrained exercisers 24 hr post-exercise. Although 3MH was not significantly different between the groups, there was a significant a 20% decrease from baseline values 24 and 48 hr post-exercise which in this study was interpreted as no effect of training status. This response could be clarified with further research since it may be possible that 3MH shows a training adaptation effect similar to that seen for CK.

An unexpected finding from this study was the increase in serum CK on the morning of day 5 for some subjects following the aerobic cycling exercise on the evening of day 4. Future research could examine the effect of consuming carbohydrate-protein, carbohydrate, or placebo supplements on glycogen resynthesis and indicators of muscle damage after this type of aerobic cycling exercise. Glycogen depletion, particularly in Type II muscle fibers, has been associated with microscopic evidence of muscle damage (Armstrong et al., 1983; Friden et al., 1988; O'Reilly et al., 1987).

A study (Zawadzki et al., 1992) that examined consumption of carbohydrate-protein, protein, or carbohydrate beverages immediately and 2 hr following cycling exercise reported significantly higher glycogen resynthesis for the carbohydrate-protein beverage (35.5 ± 3.3 μmol/g protein) over the other beverages (25.6 ± 2.3 and 7.6 ± 1.4

μmol/g protein for carbohydrate and protein, respectively). Interpretation of the results was difficult because the carbohydrate-protein beverage contained 36% and 73% more calories than the carbohydrate and protein beverages, respectively. Therefore, treatment effects could have been due to the increased caloric energy of the carbohydrate-protein beverage. A follow-up study by Tarnopolsky et al. (1997) reported consumption of isocaloric carbohydrate-protein and carbohydrate beverages immediately and 2 hr following cycling exercise resulted in similar levels of glycogen storage. A study by Cade et al. (1991) indicated the potential for post-exercise carbohydrate-protein and carbohydrate supplementation to lower serum CK after intense swim training. Interpretation of this study is somewhat difficult due to non-randomization of treatment interventions as well as supplements which varied in volume. Therefore, it would be interesting to examine the influence of post-exercise carbohydrate-protein and carbohydrate supplements on glycogen resynthesis in conjunction with indicators of muscle damage and inflammation following repeated intense aerobic exercise.

Theoretical Issues Related to Muscle Damage and Post-Exercise Nutritional Supplements

Urinary 3-Methylhistidine. An interesting finding from our study is the lower 3MH excretion seen for CP on the day of eccentric exercise when compared to C. This finding supports a recent study (Roy et al., 1997) which examined the effect of 1 g/kg carbohydrate consumption immediately and 1 hr after 80 unilateral leg extension exercise at 85% of 1-RM. They found that urinary 3MH excretion in the 24 hr following exercise was significantly lower for the supplement versus a placebo. The higher serum insulin observed for the supplement may have played a role in the reduced muscle protein degradation by 3MH (Roy et al., 1997).

We found lower 3MH excretion for CP than C on day 5 despite comparable serum insulin levels for these groups 3 hr and 6 hr post-exercise on the same day. Thus, a characteristic of the carbohydrate-protein beverage other than its influence on serum insulin appeared to affect muscle protein breakdown. One possible explanation for this finding is C also had increased serum CK on day 5 beginning with the fasted morning

value. Perhaps the damage process initiated from the cycling exercise on the evening of day 4 also concomitantly inflated 3MH excretion in this group. Nonetheless, it is an interesting possibility that a nutritional supplement consumed following novel eccentric resistance exercise may lessen muscle damage following the exercise.

There have been conflicting results regarding acute or chronic resistance exercise training on increased 3MH excretion as an indicator of muscle breakdown. One study found increased 3MH excretion by 3 days following initiation a resistance circuit exercise which persisted throughout the length of the 10 day study (Pivarnik et al. 1989). Another study that examined 3MH excretion over a 28 day resistance training program found gradual increases in 3MH over this time period (Hickson & Hinkelmann, 1985). We did not see any changes in 3MH on experimental days 6-9. It is also possible that if we extended urine collection we may have seen increases in 3MH at time points beyond four days post-exercise. The studies which found increases in 3MH used whole body circuit resistance exercise and multiple workout sessions during their experimental periods (Pivarnik, 1989, Hickson & Hinkleman, 1985). We used the quadriceps muscle group for only one exercise session. Studies which used only one or two resistance training sessions did not report changes in 3MH (Hickson, 1986, Horswill, 1988) while another study using one resistance exercise session actually found decreased 3MH excretion 24 hr following exercise (Paul et al., 1989).

Serum Creatine Kinase. The serum CK response in our subjects was highly variable, consistent with previous studies (Clarkson & Ebbeling, 1988; Costill et al., 1990; Nosaka et al., 1991; Miles et al., 1995; Nosaka & Clarkson, 1996). On experimental d 8, 3 d following the eccentric resistance exercise, 12 subjects had serum CK levels less than 100 U/L while 2 subjects had peak serum CK at approximately 10,000 U/L (Table 51). In the Costill et al. (1990) study, which utilized 8 untrained males as subjects, serum CK peaked at 6,998 ± 1,913 U/L 3 days following 100 repetitions of eccentric quadriceps resistance exercise. The highest value reported was 30,196 U/L, and 2 other subjects had serum CK over 8,000 U/L at this time point (Costill et al., 1990). Increases of CK to less than 125 U/L were reported in 2 non-weight trained

female subjects by Miles et al. (1995), but one subject demonstrated CK levels of 45,670 U/L in the days following their performance of 50 repetitions of unilateral eccentric biceps and quadriceps resistance exercise.

It is possible that intensity of effort by the subjects or variable amounts of work performed during the eccentric exercise may have influenced our CK results, but this presumption cannot be determined from the information available in the other studies (Clarkson & Ebbeling, 1988, Costill et al., 1990; Nosaka et al., 1991; Miles et al., 1995; Nosaka & Clarkson, 1996). A recent study reported a low serum CK response following eccentric resistance exercise was associated with microscopic evidence of muscle damage. Hortobagyi et al. (1998) reported no increases in serum CK (range of 31-42 U/L) when a bout of 100 repetitions of eccentric quadriceps exercise was repeated 2 weeks following an initial bout that elicited 220% increases in CK in 18 non-weight trained subjects. However, some subjects continued to demonstrate microscopic evidence of muscle damage that included disorganized myofilaments and Z-line streaming following the second bout of exercise. Therefore, it is possible that a low serum CK following eccentric exercise may not necessarily translate to no microscopic evidence of muscle damage.

An interesting finding from our study was that 8 subjects demonstrated increased serum CK prior to eccentric exercise on d 5 in comparison to the morning of d 4. This suggests the high-intensity cycling exercise the night before the eccentric resistance exercise initiated some muscle damage in these untrained subjects. This is possible because high-intensity concentric exercise has been associated with microscopic evidence of muscle damage (Armstrong et al., 1983; Friden et al., 1988). An analysis of covariance with serum CK on day 5 morning as the covariate did not alter the statistical results. However, the issue remains that the cycling exercise may have been of sufficient intensity to initiate a serum CK and inflammatory response in these untrained subjects. Therefore, the time course of CK response from the eccentric resistance exercise in these subjects may have been altered in these subjects relative to studies that did not include intense aerobic exercise combined with eccentric resistance exercise. Studies which

examine eccentric aerobic exercise such as downhill running often report peak serum CK values at 24 hr post-exercise (Byrnes et al., 1985; Evans et al., 1986). On the other hand, studies which examine high force eccentric exercise utilizing resistance training report peak serum CK values 3-5 days post-exercise (Clarkson & Ebbeling, 1988, Ebbeling & Clarkson, 1989; Nosaka et al., 1991; Nosaka & Clarkson, 1996). Furthermore, the peak serum CK responses are often less in downhill running when compared to high force eccentric exercise (Byrnes et al., 1985; Clarkson et al., 1992; Evans et al., 1986).

Although many studies involving novel eccentric exercise have found a several-hundred-fold increase in serum CK response (Costill et al, 1990, Clarkson & Tremblay, 1988; Nosaka et al., 1991; Nosaka et al., 1992; Newham, 1987), there have been other reports of high-force eccentric exercise eliciting only modest increases in serum CK responses. In contrast to the study by Costill et al. (1990) where aerobic cycling followed eccentric resistance exercise, a subsequent study from the same laboratory (Widrick et al., 1992) did not find a significant increase in CK response when eccentric resistance exercise followed aerobic cycling exercise. Pre-eccentric exercise CK values in this study (Widrick et al., 1992) were somewhat elevated (227 ± 75 U/L) over normal values of less than 100 U/L (Sigma Chemical Co., St. Louis, MO). As a result, perhaps some muscle damage was already initiated in these subjects from the exhaustive cycling exercise performed the evening before. Damage induced by this cycling protocol may explain the modest CK response following the eccentric resistance exercise several days later. Using a similar protocol where 100 eccentric leg flexions immediately followed aerobic cycling and cycling sprints, Doyle et al. (1993) reported significant increases in CK responses from their baseline values. However, their peak CK values 72 h post-exercise were only moderately elevated to 444 ± 157 U/L from higher-than-normal pre-exercise values of 147 ± 23 U/L.

The subject characteristics in the above studies may have influenced the CK response. The study which found the greatest increase in serum CK using a protocol which combined eccentric resistance exercise with cycling exercise (Costill et al. 1990) utilized subjects who were not involved in any eccentric activity while other studies that

utilized subjects who were endurance trained but not performing resistance exercise involving their leg musculature found smaller increases in serum CK (Doyle et al., 1993; Widrick et al., 1992). Several studies have shown that prior eccentric exercise can blunt the effect on the serum CK response to subsequent eccentric exercise. This adaptation develops rapidly in the days following exercise--even before the serum CK returns to normal levels--and can last for up to six months (Clarkson & Ebbeling, 1988; Clarkson & Tremblay, 1988; Hortobagyi et al., 1998; Nosaka et al., 1991). The mechanism for this rapid adaptation remains unclear at this time but may include both cellular and neural adaptations (Clarkson et al., 1992). It is possible damaged and necrotic fibers are near the end of their life span and are replaced by stronger fibers (Armstrong et al., 1983). Also, neural factors may be involved with the CK training adaptation because the adaptation begins before recovery of other indicators of muscle damage such as muscle strength and flexion/relaxation ability of the joint around the involved muscle group (Ebbeling & Clarkson, 1990). In addition, the intensity of the eccentric exercise required to elicit this protective response need not be very high. Performance of resistance exercise with as little as 12 repetitions or performance of any unaccustomed activity such as lifting boxes has potential to elicit this CK adaptation response (Clarkson et al., 1992; Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1989). Thus, individuals involved in any type of regular activity that may include some downhill running could perform some unaccustomed activity involving eccentric contractions of the thigh muscles that could influence their serum CK response. The recreational activities performed by subjects in this study may have contributed to the variability in the CK response. Also, exercise such as the 1-RM testing could have been of sufficient intensity in some subjects to affect their serum CK response.

Muscle Soreness. Along with the increased serum CK response, eccentric resistance exercise is associated with the development of significant muscle soreness which typically develops within 24 hr and peaks about 72 hr post-exercise (Costill et al., 1990, Doyle et al., 1993; Nosaka & Clarkson, 1986; Smith et al., 1994). We were able to elicit significant muscle soreness which increased over day 5 and peaked approximately 3-4 days post eccentric resistance exercise, but we were unable to influence muscle

soreness at any of these time points as a result of beverage consumption post eccentric resistance exercise. The subjects in our study reported a moderate degree of soreness consistent with values reported in other similar studies (Doyle et al., 1993). Maximal soreness for each treatment group occurred on day 7 and was 3.4 ± 0.5 for C, 4.3 ± 0.8 for CP, and 4.1 ± 0.5 for P and was not affected by beverage consumption. These responses were widely variable among the individuals and ranged from 1 (no soreness) to 8 (greater than very strong soreness) (See scale in Appendix J and raw data in Table 52). Like serum CK, the soreness response can demonstrate an adaptation effect in that prior eccentric exercise can diminish soreness in subsequent bouts of eccentric exercise (Ebbeling & Clarkson, 1990; Clarkson et al., 1992; Hortotagyi et al., 1998).

Muscle Function. About a 50% loss of muscular force production is typically seen immediately following eccentric resistance exercise (Ebbeling and Clarkson, 1990; Newham et al., 1987; Nosaka et al., 1991; Nosaka & Clarkson, 1996). This decreased muscle function persists for several days and gradually returns to normal by 10-12 days post-exercise (Newham et al., 1987; Clarkson et al., 1992). Our eccentric resistance protocol evoked significant decreases in isokinetic peak torque, work done with the maximal repetition, and total work for five repetitions at 24 hr post-exercise which slowly began recovery at 48 and 72 hr post-exercise (Figure 4). Post-exercise beverage consumption did not affect muscle function from 24-72 hr post-exercise. Although researchers have attempted to link decreased muscle function to muscle soreness, the muscular deficits persist after muscle soreness disappears by 5-7 days following exercise (Newham et al, 1987; Clarkson et al., 1992).

Serum Cortisol. Glucocorticoids such as cortisol are associated with anti-inflammatory effects in tissues which include decreased migration of inflammatory cells such as monocytes and lymphocytes to sites of injury or trauma as well as inhibition phospholipase A_2 to limit prostaglandin synthesis (Greenspan & Baxter, 1994). Also, excess glucocorticoids within and above normal physiological values are associated with muscle wasting by mechanisms of both stimulating protein breakdown and inhibiting protein synthesis in muscle (Capaccio et al., 1987; Simmons et al., 1984). Previous

studies which emphasized concentric resistance training reported increases in serum cortisol in untrained men immediately and one hour following squatting exercise that ranged from 40-90% 1-RM (McMillan et al., 1993) and immediately and one hour following a whole-body resistance training circuit performed at 70% 1-RM (Jurimae et al., 1990).

The 24% drop in serum cortisol we saw across all groups 1-3 days following the eccentric exercise (experimental days 6-8) is in contrast to a recent study (Nosaka and Clarkson, 1996) which found no changes in morning serum cortisol 5 days post-exercise in subjects who performed 24 eccentric contractions of the elbow flexors. Post-exercise nutrient consumption did not influence morning serum cortisol. Although our study found statistically significant changes in morning serum cortisol, group means were still within normal physiological ranges of 50-200 ng/ml (Greenspan & Baxter, 1994). Levels of serum cortisol associated with trauma, stress, and injury are typically 400-600 ng/ml (Greenspan & Baxter, 1994). Serum cortisol levels are sensitive to emotional stress, and both increases and decreases of cortisol have been detected due to anticipation of laboratory testing or athletic competition (Viru, 1985).

Since serum cortisol exhibits circadian rhythm with levels highest in morning, it was not surprising to find serum cortisol fell 50% over day 5 (Miles et al, 1995, Smith et al., 1994). In addition, all our subjects experienced a similar serum cortisol levels at three and six hours post-exercise which demonstrated no effect of treatment beverage on serum cortisol.

Practical Implications of Post-Exercise Nutritional Supplements and Muscle Damage

A carbohydrate-protein beverage (5 kcal/kg or 0.875 g CHO/kg, 0.375 g protein/kg) consumed immediately and two hours following eccentric resistance exercise has potential to affect indicators of muscle damage and muscle protein breakdown in the immediate hours following exercise. Lowest changes in serum creatine kinase on the day of exercise were observed for the carbohydrate-protein beverage. Furthermore, urinary 3-methylhistidine excretion was lowest for the carbohydrate-protein beverage in the first

24 hr following eccentric resistance exercise, indicating less muscle protein breakdown. This effect may be mediated through enhanced serum insulin levels following consumption of the carbohydrate-protein beverage. Therefore, persons performing resistance training may wish to consume a carbohydrate-protein beverage following a bout of intense or novel eccentric resistance exercise.

Theoretical Issues Related to Muscle Glycogen Resynthesis and Post-Exercise Nutritional Supplements

This study suggests that consumption of a carbohydrate or carbohydrate-protein beverage following eccentric resistance exercise has potential to facilitate a hormonal environment temporarily more favorable for muscle glycogen and protein synthesis. Typically, consumption of a carbohydrate alone or in combination with protein stimulates the β cells of the pancreas to release insulin which facilitates entry of glucose and amino acids into the muscle cells (Nuttal et al., 1984; Spiller et al., 1987). The serum insulin responses in our study for both CP and C at 3 hr post-exercise were similar to each but different from P. These responses at 3 hr post-exercise support those seen by Chandler et al. (1994) who examined hormonal responses after consumption of carbohydrate-protein, carbohydrate, and protein supplements or water following a resistance circuit training exercise. In their study, the carbohydrate-protein and carbohydrate beverages consumed immediately and 2 hr post-exercise produced significantly higher serum insulin levels versus protein and placebo at 1 hr post-exercise and significantly higher than placebo at 3 hr post-exercise. Thus, higher serum insulin observed for CP and C in the post-exercise period for both studies can be interpreted as favorable hormonal environment for muscle growth.

At 24 hr post-eccentric exercise, skeletal muscle glycogen levels were somewhat lower than normal resting values across all treatments, which is consistent with other studies (Costill et al., 1990). However, there was no benefit of consuming either CP or C beverage relative to the P beverage on muscle glycogen 24 hr following eccentric resistance exercise. Since there was no significant difference in total energy on day 5

between treatment beverage groups, the total energy on this day may help explain the similar glycogen levels observed across all groups at 24 hr post-exercise. Therefore, overall energy on day 5 may have overcome the effects of the treatment beverages by 24 hr post-exercise.

It is possible that the higher serum insulin levels for CP and C versus P at 3 hr could have influenced the rate of skeletal muscle glycogen resynthesis in the more immediate hours following exercise. A study by Roy & Tarnopolsky (1998) examined muscle glycogen resynthesis for carbohydrate/protein/fat, carbohydrate, and placebo beverages consumed immediately and 1 hr following a resistance circuit exercise session. Both treatment beverages in their study produced comparably elevated serum insulin levels for 3 hr post-exercise that were significantly different from the placebo. As a result, the muscle glycogen resynthesis taken from biopsies at 4 hr post-exercise was significantly higher for the treatment beverages versus the placebo and control muscle from the non-exercised contralateral leg (Roy & Tarnopolsky, 1998). Thus, future studies examining the effect of post-exercise carbohydrate-protein beverages should take muscle biopsies earlier than the 24 hr time point in our study.

Serum Testosterone. Testosterone is the steroid hormone associated with increased muscular hypertrophy. It is believed to initiate increased protein synthesis in muscle following binding with a specific androgen receptor (Capaccio et al., 1987). The changes observed in our study for morning serum testosterone, although statistically significant, probably have little physiological importance since the change in serum testosterone was only a 7% decrease from experimental days 4-5. These values were well within normal ranges of 3-10 ng/ml (Greenspan and Baxter, 1994). Furthermore, we were not able to influence morning serum testosterone with post-exercise nutrient consumption.

Serum testosterone was not acutely affected by the eccentric resistance exercise. Other experimental protocols elicited an increase in serum testosterone following heavy resistance exercise (Jurimae et al., 1990; Volek et al., 1997), but this response may be related to a threshold intensity of exercise, volume of the training program, or amount of

muscle mass exercised (Kraemer, 1988). Our eccentric exercise protocol may not have been of sufficient intensity, volume, or muscle mass to elicit increased serum testosterone immediately post-exercise in our subjects.

At 3 hr post-exercise on day 5, lower serum testosterone was observed for those subjects who consumed either nutrient-containing beverage compared to the placebo. These results corroborate those obtained by Chandler et al. (1994) who found lower serum testosterone from 2-5 hr post-resistance circuit exercise for carbohydrate, protein, and carbohydrate-protein supplements versus placebo. Measurement of luteizining hormone during the carbohydrate trial showed no changes during these same time points. Therefore, they hypothesized that increased clearance of serum testosterone instead of reduced secretion may be responsible for the lower serum testosterone observed following consumption of their supplements. A recent study (Volek et al., 1997) which examined 17 days of diet records from males who performed 5 sets of 10-RM bench pressing and 30% 1-RM jump squats reported significant negative correlations between serum testosterone and dietary protein ($r = -.71$) and protein/carbohydrate ratio ($r = -.59$). Their findings suggest macronutrient-based alterations in either metabolism of testosterone or production of sex hormone binding globulin. Yet, it is interesting to note that manipulation of macronutrients following resistance training exercise may influence the serum testosterone response. The implications of altered post-exercise serum testosterone responses on muscle protein synthesis remain to be clarified for persons undergoing resistance training.

Practical Implications of Post-Exercise Nutritional Supplements and Muscle Glycogen Resynthesis

Skeletal muscle glycogen levels 24 hours following eccentric resistance exercise were not affected by consuming a carbohydrate (5 kcal/kg or 1.25 g CHO/kg), carbohydrate-protein (5 kcal/kg or 0.875 g CHO/kg, 0.375 g protein/kg), or placebo supplements immediately and 2 hr following eccentric resistance exercise. However, carbohydrate and carbohydrate-protein supplements can promote a hormonal environment temporarily more favorable for muscle glycogen resynthesis as well as for

muscle contractile protein synthesis, an effect that is likely mediated through elevated serum insulin levels in the hours post-exercise. Therefore, persons performing intense resistance training may wish to consume either a carbohydrate or carbohydrate-protein supplement following exercise to help restore skeletal muscle glycogen, especially if they need to restore muscle glycogen before 24 hr post-exercise.

Overall Conclusions

The aim of this project was to evaluate the effect of consuming carbohydrate, carbohydrate-protein, or placebo beverages immediately and two hours following eccentric resistance exercise on indicators of muscle damage, muscle contractile protein resynthesis, and muscle glycogen resynthesis. Consumption of a carbohydrate-protein beverage following eccentric resistance exercise may be indicated to lessen contractile protein breakdown and may be as effective in promoting a hormonal environment for muscle glycogen resynthesis as a carbohydrate beverage. The potential for post-exercise macronutrient manipulations and their effect on muscle damage, muscle glycogen, and muscle protein synthesis remain an interesting area of further investigation.

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Appendix A

Detailed Methodology

Methods

Subjects

Approximately 175 males responded to classified advertisements in the campus newspaper as well as to posted flyers in the campus student center, gym, and engineering and business classroom buildings. The advertisements and flyers requested males age 18-30 years old to participate in a 10-day study on nutrition and exercise that involved multiple blood draws and 3 muscle samples taken from the thigh. No selection criteria were listed in the advertisement or on the flyer so that any interested persons would be honest during screening. All of these males underwent a preliminary screening (Appendix D) over the phone or via electronic mail to see if they met selection criteria which included the following: (a) Untrained fitness status with particular emphasis on no running or resistance training involving the legs for at least 6 months, (b) no medical contraindications such as chronic disease or orthopedic limitations, (c) no lactose intolerance or milk allergy, (d) no allergy to local anesthetics, (e) no apparent difficulties during blood draws, and (f) availability during the proposed data collection periods.

All potential subjects who remained qualified following the initial screening were invited to an informational session in one of the large lecture hall classrooms on campus. At this time, each experimental day and procedure of the study was described in detail, and persons present had the opportunity to ask questions. Those who remained interested were asked to give their names and when they believed they could participate in the study. These males were contacted to fill out a more detailed medical and exercise history form which included their class and work schedule in order to determine their availability during data collection time periods (Appendix E).

Based on criteria from the medical and exercise history form, 27 males were chosen to participate in the study. They were randomly assigned to receive the carbohydrate-protein, milk-based beverage (CP, n = 9), the carbohydrate beverage (C, n =9), or the placebo beverage (P, n =9) on experimental day 5. One subject in the CP

group was asked to withdraw participation from the study because of syncope (fainting) during a blood draw. Therefore, 26 subjects completed the study, and the CP group contained only 8 subjects. It was not possible to add another subject to the CP group because the syncopal event occurred during the final wave of data collection.

These subjects were scheduled in waves of 2-5 subjects beginning every Thursday and ending 10 days later on Saturday morning (Appendix F). Approximately one week prior to their experimental wave period, subjects reported to the laboratory to sign the informed consent form (Appendix C) and undergo pre-experimental tests.

Body Weight and Height

Each subject was asked to wear shorts and remove shoes. Body weight was recorded to the nearest 0.1 kg using a medical balance-type scale. Height was recorded to the nearest 0.1 cm when the stadiometer portion of the scale was horizontal on the subject's head while he stood with his back to the scale (Appendix G).

Percent Body Fat

Body composition was assessed using Harpenden skinfold calipers (Country Technology, Gays Mills, WI) and the protocol of Jackson and Pollack (1985). All measurements took place on the right side of the body. The three sites used for measurement in this protocol were the chest, abdomen, and thigh (Appendix G). The chest site was a diagonal skinfold midway between the nipple and the anterior axillary fold. The abdominal site was a vertical skinfold located 2 cm to the right of the umbilicus. The thigh fold was a vertical skinfold midway between the proximal border of the patella and the inguinal crease. Each site was measured to the nearest 0.1 mm, and the sites were rotated to prevent compression of subcutaneous fat. The average of 3 values within 1 mm were averaged and used in a regression formula to determine percent body fat (Jackson and Pollack, 1985).

Biodex Performance Test

Quadriceps peak isokinetic torque, total work for five repetitions, and work performed with the maximal repetition were determined using a Biodex System II isokinetic dynamometer (Biodex Corp., Shirley, NY). The dominant leg was used, which was the limb of choice to kick a ball. All subjects were given a familiarization trial since pilot testing of the isokinetic protocol revealed a learning effect that stabilized with a second trial. The baseline isokinetic data was collected on a separate day.

Prior to each testing session, the subject performed a 5 min warm-up on a cycle ergometer (Monarch 818E, Stockholm, Sweden) using a light resistance of 0.5 kg on the flywheel. The subject was then seated on the chair portion of the dynamometer. The axis of rotation of the dynamometer was lined up with the dominant leg medial femoral epicondyle. This was accomplished by adjusting the forward-backward motion of the seat, the angle of the chair back, and the vertical dynamometer position. Also, the lever arm of the axis of rotation was determined by placing the bottom portion of the shin pad approximately 2 cm above the dorsum of the foot to allow the foot to remain flexed. Range of motion was determined by having the subject fully extend and fully flex his leg. All these measurements were recorded for each subject and used again for all his subsequent testing sessions (see recording form in Appendix H).

Use of body momentum during testing was minimized by using Velcro^{TM} stabilizer straps over the shin pad, across the hips, and crossing the shoulders. The stabilizer strap over the mid-thigh was not used since it was found during pilot testing that this strap would be placed directly over the thigh area where the muscle biopsies occurred. Therefore, this strap was not used in any experimental subjects in order to avoid irritation of the muscle biopsy site. Furthermore, to minimize body momentum during testing, all subjects kept their forearms crossed over their chests with the hands touching the opposite shoulders.

The testing protocol consisted of five maximal extension-flexion repetitions beginning from the point of maximal flexion. The testing speed was 60^{o}/second. At the command "One, two, three, Go!", the subject was encouraged to give a maximal effort for every repetition for both directions of movement. Consistent maximal effort could be determined by examining the consistency of torque curves, which appeared on the computer monitor attached to the Biodex. No count of repetitions was given to the subject, and the subject was not allowed to see the computer monitor. The Biodex beeps and stops collecting data after the five repetitions, and the subject was then told to relax. A one-min rest period was given between sets.

At least two trials were performed for each testing session. If the coefficient of variation for one trial was greater than 15%, another trial was performed. Quadriceps peak torque, total work for the five repetitions, and work done with the maximal repetition were obtained from printouts. The mean values for the two trials were used in statistical analysis.

<u>One-Repetition Maximum (1-RM) Testing</u>

The subject's dominant leg also had to undergo 1-RM testing, the maximal amount of weight the subject can lift with the leg for one repetition. The resistance used for the eccentric exercise protocol will be 120% of 1-RM determine from this test. Testing was performed on a Nautilus isotonic leg extension station (Nautilus, Independence, VA) located in a weight room in the athletics building.

Optimal subject position for the test was obtained by using Velcro straps to secure the hips to the chair and by using pads to adjust seat-back position. The rest of the machine is not as adjustable as an isokinetic dynamometer. Momentum was minimized by having the subject keep his forearms crossed as with the Biodex testing. The subject was encouraged to give a maximal effort and to exhale as he gave the effort. One minute of rest was given between trials. One-repetition maximum was determined by increasing or decreasing the resistance on the weight stack until the subject could only perform one

repetition through full range of motion at a given weight (Appendix H). A 2.27 kg (5 lb.) adapter was added to the weight stack to enhance precision.

Aerobic Capacity Testing

Approximately seven days prior to the experimental period, following the performance test subjects underwent testing for peak oxygen uptake (VO_{2peak}) on a stationary cycle ergometer (Monarch 818E, Stockholm, Sweden). At the time the subjects were present for percent body fat testing, they were given a familiarization trial to set up seat and handlebar heights on the cycle ergometer. This information was recorded so that it could be used for the data collection (Appendix H). The subjects also were able to practice pedaling the cycle to a cadence 60 revolutions per minute (RPM) set by a metronome at 120 beats per minute. In addition, the subjects were able to become familiar with the mouthpiece and noseclip that would be used in the testing procedure. They were also taught how to signal the testing personnel while the mouthpiece was in place. Finally, the subjects were instructed in how to report ratings of perceived exertion (RPE) according to the Borg 6-20 scale (Borg, 1982; Appendix H). Two RPE readings were taken--one for overall feeling and another reading for just the legs. The scale was explained to them that a rating of 6 was equivalent in intensity to sitting on a couch while a rating 20 would be the hardest physical work they have ever performed.

For the actual test, subjects reported to the laboratory either after an overnight fast or at least 4 hr following a meal. The instruction form given to the subjects in preparation for the test can be found in Appendix H. The subject was re-weighed in shorts and with no shoes. Small quarter-sized patches of skin in the areas of both subclavicular fossa and inferior to the left inferior intercostal border were wiped with a rubbing alcohol pad. In order to remove dead skin and surface oil for better electrode contact, the skin was then lightly scrubbed in a circular motion until it was light pink using a sterile gauze pad. The pre-gelled electrocardigraphic electrodes (BioTac, Graphic Controls Corp., Buffalo, NY) were then placed over these three areas. The wires to the electrocardiogram (Lifepak 9, Physio-Control Corp., Redmond, WA) were snapped onto the electrodes, and the subject

was escorted to the cycle which was already set to his previously determined seat and handlebar heights. Finally, the mouthpiece and noseclip were put in place.

The indirect calorimetry metabolic cart (Model CPX/D, Medical Graphics Corp., St. Paul, MN) was calibrated 30 min prior to each test using room air as well as a calibration gas consisting of 18% oxygen and 6% carbon dioxide mixture. If multiple tests were performed on the same day, the cart was recalibrated between each test. The subject's age, height, and current weight were entered into the system prior to the start of the test. Oxygen uptake was calculated using breath-by-breath sampling of expired oxygen and carbon dioxide as well as ventilation volume. Lightweight plastic tubing connected the mouthpiece to the metabolic cart.

Two minutes of resting data were collected. The subject began pedaling at 60 RPM. The initial workload on the cycle was 0.5 kg, and the workload was increased by 0.5 kg following each 2 min stage. The subject was told when the next increment would occur. Heart rate was recorded from the electrocardiogram at the end of every minute although it was continuously monitored for rate and rhythm. RPE data for overall and legs were collected at the end of each stage. The test was terminated when the subject could no longer maintain pedaling frequency despite encouragement. RPE measurements were also taken at this time. The time of test termination was recorded. Following completion of the test, the resistance on the cycle was turned back to 0.5 kg, and the subject was given a 4 min recovery and monitored. Once the recovery period was over, the mouthpiece and noseclip were removed, and the subject was assisted from the cycle and unhooked from the electrocardiogram. Peak VO_2 was calculated as the average of the values over the final minute of exercise obtained from the printout.

Overview of Experimental Design

The experimental period consisted of 9 full days plus the morning of day 10 (Figure 1). All subjects underwent a controlled feeding for days 1-9. The subjects performed 24-hr urine collections on days 3-9. Fasted blood samples were obtained the

mornings of days 4-10. All subjects underwent quadriceps muscle glycogen depletion exercise bout using a cycle ergometer on the evening of day 4. A low carbohydrate snack followed the exercise to keep muscle glycogen reduced. On the morning of day 5 fasted subjects performed 100 eccentric leg flexions of the quadriceps. Additional blood draws were obtained immediately, 3 hr, and 6 hr following the eccentric exercise. Muscle biopsies from the vastus lateralis were obtained immediately, 24 hr, and 72 hr post-eccentric exercise. Each subject was given the assigned treatment beverage (CP, C, or P) following the muscle biopsy and 2 hr later. Quadriceps muscle soreness was assessed on a 1-10 scale while the subject was resting quietly at the time of each blood draw on day 5 as well as the mornings of days 6-10. Quadriceps peak isokinetic torque, total work for 5 repetitions, and work performed with the maximal repetition were assessed pre-experimentally as well as 24, 48, and 72 hr post-eccentric exercise.

Body Weight

Body weight was monitored to the nearest 0.1 kg every morning using a medical scale. All subjects removed shoes prior to weighing in (Appendix G).

Dietary Control

The controlled diet consisted of a meat-free, 3-d rotating menu with a macronutrient content of 55 % carbohydrate, 30% fat, and 15% protein. A meat-free diet is indicated for measuring urinary 3-methylhistidine (3MH). Since 3MH is a an amino acid component of skeletal muscle, consumption of meat products can erroneously inflate 3MH values (Thomas, Ballard, and Pope, 1979;Young and Munro, 1978).

The menus were developed by a departmental research associate who had prior experience in controlled feedings and were based upon the protocols of Hickson et al. (1986). Dietary menus were constructed using Nutritionist IV software (First Data Bank, San Bruno, CA). Each day's menu consisted of a breakfast, lunch, and dinner at fixed times with snacks that could be eaten at flexible hours throughout the day (Appendix I).

Meals were prepared in a foods teaching classroom by the research associate or other trained foods personnel in the Department of Human, Nutrition, Foods, and Exercise. All food and beverage portions were measured using measuring cups, measuring spoons, or an electronic balance scale. All food personnel used gloves during preparation, serving, and cleanup. Since the subjects were not housed overnight on campus, they ate all of their breakfasts and most of their dinners in the teaching classroom and returned home each night. For any other meals not consumed in the classroom, the subjects received a small, insulated cooler packed with their pre-measured dinner, lunch, or snacks.

Initial energy of the diet was set at 3,000 kcal/day. Subjects who experienced small weight losses and/or expressed hunger were supplemented with Ensure liquid supplement (Abbott Laboratories, Columbus, OH) since it provided additional kcal while maintaining the macronutrient breakdown of the controlled diet. Subjects were instructed to consume no other foods, vitamin or mineral supplements, or alcoholic beverages during the experimental period. Diet record check-off sheets were turned in on a daily basis, and any Ensure consumed or food not eaten was accounted for. All of these subjects' diet records were analyzed for caloric and macronutrient content using Nutritionist IV software.

On the day of the eccentric resistance exercise (day 5), diets were adjusted in kcal and macronutient content to account for the experimental beverages so that the original caloric (3000 kcal/day) and macronutrient content (55 % carbohydrate, 30% fat, and 15% protein) were maintained. Also, the timing of meals was adjusted to accommodate the treatment beverages consumed that morning. A brunch meal was the first solid food consumed 3 h post-exercise following a blood draw (Appendix I).

Urine Samples

Subjects completed 24 hr urine collections for experimental days 3-9. Samples were collected in 1.0 L polypropylene bottles with 1 ml 50% hydrochloric acid as a preservative. Collections began with the second void of the day and were turned in each

morning. The subject's daily samples were carefully mixed, total volume was recorded (Appendix L), and 5 ml aliquots were frozen at -20^{o}C.

Blood Collection

Blood samples (10 ml) from the antecubetal vein were collected by a certified clinical laboratory technician. Fasting blood samples were collected on the mornings of days 4-10. Additional tubes of blood were taken on the day of eccentric exercise immediately, 3 hr, and 6 hr post-exercise. All samples were placed in an ice bath for 30 min to clot and were centrifuged at 3,000 rpm for 15 min at 4^{o}C. Aliquots of serum were stored at -20^{o}C until analysis. All samples for a subject were analyzed in duplicate during the same run of the assay.

Aerobic Glycogen Reducing Protocol

The purpose of this protocol was to reduce skeletal muscle glycogen since several research groups found evidence that glycogen-depleted muscle could be more susceptible to damage from eccentric contractions (O'Reilly et al., 1987; Sherman et al., 1983). The protocol was based on the methods of Doyle et al. (1993), Widrick et al., (1992), and Costill et al., (1990).

The subjects arrived in groups of 2-3 at the laboratory 3 hr following their dinner on day 4. They were once again prepped for and hooked up to the electrocardiographic telemetry as they were during the maximal aerobic capacity test. The cycle ergometer seat and handlebar settings were the same as the aerobic capacity test. The resistance on the cycle ergometer flywheel was set as the resistance corresponding to 70% of VO_{2peak}. Individual time clocks were set for each subject. Metronomes set the pedaling cadence at 60 rpm (see testing form in Appendix H).

During the exercise session, subjects were allowed to drink water ad libitum. Heart rate and RPE were recorded every 5 min. A 2 min rest period was allowed if the

subject requested, but all subjects completed a total exercise time of 40 min. Following the 40 min aerobic exercise session, the subjects completed 5 bouts of 1 min maximal cycling sprints in order to further reduce skeletal muscle glycogen. Cycle resistance for the sprints was kept the same as the aerobic cycling protocol, and subjects were encouraged to put forth an all-out effort. A 2 min active recovery on the cycle with a resistance of 1.0 kg followed each sprint. At the end of the exercise, subjects performed and active cooldown for 5 min and were observed for any signs of dizziness or lightheadedness.

Low Carbohydrate Snack

By the time the subjects arrived at the laboratory for the aerobic glycogen reducing protocol, they were told they must have consumed all food for the day except for the low-carbohydrate snack. The snack consisted of 36 g roasted peanuts plus 1 hard-boiled egg and provided a total energy content of 290 kcal. The macronutrient breakdown of the snack was 10% carbohydrate, 21% protein, and 69% fat. The rationale for this snack was to keep the subjects' glycogen stores low until they performed the eccentric resistance exercise the following morning.

Muscle Soreness Protocol

At the time of each blood draw on day 5 (fasting, immediate, 3 hr, and 6 hr post-exercise) and at the morning blood draws on days 6-10, the subjects were asked to rate their overall muscle soreness in their dominant leg quadriceps muscles using a 1-10 scale (1 = no soreness, 10 = unbearable soreness). The subjects were asked to rate their overall soreness in their quadriceps while they were resting following the blood draw. The scale was taken from the methods of Clarkson et al. (1986). A sample of the scale can be found in Appendix J.

Eccentric Resistance Exercise Protocol

This protocol was also based on the methods of Costill et al., (1990), Doyle et al., (1993), and Widrick et al. (1992). Following a fasting blood draw and body weight measurement, subjects reported to the small weight training room in the athletic facility. The subject sat in the leg extension station, and the settings were adjusted to those used for the 1-RM testing. The hips were secured with a strap, and the subject kept his arms crossed across the chest. A sample of the form is included in Appendix H.

The weight stack was set to 120% of the 1-RM which was determined in the pre-experimental period. Several research assistants raised the weight stack and placed the lever arm pad on the subject's shin. The subject was then asked to lower the weight stack for a 4 s count to a metronome set at 60 beats/min. In order to prevent a Valsalva maneuver, each subject was asked to exhale as he lowered the weight stack. All subjects completed 10 sets of 10 repetitions of eccentric leg flexions with 1 min rest between sets. Fatigue occurred during the protocol, and in the last few sets the subjects had difficulty maintaining the 4 s contraction time. Following the last set of exercise, the subject was assisted from the leg extension machine and escorted into the training room.

Muscle Biopsy Procedure

The subject was assisted to a semi-reclined position on a training room table. He was not able to see the procedure from this position. A sterile environment was maintained by using a sterile drape, autoclaved instruments, and sterile surgical gloves.

The site for the incision was located on the outer portion of the thigh over the vastus lateralis muscle, and the skin was cleaned with a topical antiseptic solution. The skin was then cooled with a topical solution of ethyl chloride. Following this, xylocaine was administered in several punctures under the skin. When the xylocaine took effect after several minutes, a scalpel was used to make the incision over the previously marked site through the fat, fascia, and into the muscle.

The hollow biopsy needle was inserted into the incision. A second technician applied suction, which brings some of the muscle into the hollow window of the needle. An inner portion of the needle was slid down to cut the muscle. The needle was then pulled out of the thigh.

The muscle biopsy sample, approximately 50 mg, was removed from the needle, weighed on an analytical balance, placed in a cryovial, and immediately frozen in liquid nitrogen. The cryovials were removed from the liquid nitrogen at the laboratory, and all muscle biopsy samples were stored at -80^{o} C until analyses.

Treatment Beverages

Following the muscle biopsy procedure and 2 hr later, each subject was given his randomly assigned CP, C, or P beverage. The subjects were blinded as to which beverage they received.

All beverages were made as 13% solutions and provided 5 kcal/kg bw (Chandler et al., 1994; Pascoe et al., 1993) (Appendix K). The CP beverage consisted of skim milk sweetened with extra carbohydrate from strawberry-flavored Nestle Quik (Nestle Corp., San Francisco, CA) and provided 5 kcal/kg in the form of 0.875 g carbohydrate/kg, 0.375 g protein/kg. Selection of the carbohydrate/protein ratio of the CP beverage was based on studies performed by Chandler et al. (1994) and Spiller et al. (1987). The C beverage provided 1.25 g carbohydrate/kg (5 kcal/kg) from orange-flavored Gatorade (Quaker Oats Co., Chicago, IL). The P beverage consisted of an aspartame-flavored drink that provided approximately 16 kcal/L (Crystal Light, Kraft Foods, Inc., White Plains, NY). All powders were measured using an electronic balance, and all fluid volumes were measured using graduated cylinders.

Laboratory Procedures

Cortisol Radioimmunoassay Procedure.

1. Label four plain 12 x 75 mm polypropylene test tubes (#14-956-1B, Fisher Scientific, Pittsburgh, PA) T (for total counts) and NSB (nonspecific binding) in duplicate.

2. Label 12 cortisol antibody-coated tubes (Diagnostic Products Corp., Los Angeles, CA) A-F in duplicate for calibrators. Label antibody-coated tubes for subject samples starting with number 17 in duplicate. Label key to associate subject samples with respective tube number.

3. Pipette 25 μl of the A calibrator in duplicate into the NSB and A tubes. Pipette 25 μl of each B-F calibrator in duplicate into its respective tube. All samples should be pipetted directly to the bottom of the tube.

4. Pipette 25 μl of each subject sample, thawed at room temperature, in duplicate into its appropriate tube. All samples should be pipetted directly to the bottom of the tube. Change pipette tips following the duplicate samples. Use gloves and follow universal precautions against blood-borne diseases.

5. Add 1.0 ml of ^{125}I Cortisol to every tube using a measuring pipette designated for isotope use. Dispensing of the tracer should take no more than 10 minutes. Gently vortex each tube. Survey gloved hands and working area with Geiger counter.

6. The T-tubes should be set aside under the hood since they require no further processing until counting.

7. Cover the rack of tubes with aluminum foil. Place the tubes in a water bath heated to 37^{o} C. Incubate for 45 minutes.

8. After 45 minutes, remove the tubes and take back to the hood to decant. Fill the pans labeled “Waste” and “Rinse” about 25% full with water and place in hood. Place a mesh rack over the test tube rack, keeping hands at the ends. Gently invert the rack to drain into the waste pan. Next, place the rack into the rinse pan. Tap out any excess water. Turn over the rack and leave in hood on tray covered with absorbent paper. Gently slide of the mesh rack. The tubes may be left in the hood to air dry before counting. All radioactive waste is placed into the large waste container in the hood. The waste pan can be rinsed in the hood. Both pans can then be decontaminated and cleaned in a sink designated for cleaning. Survey hands and all areas.

9. Place all tubes (T tubes included) in numerical order in the scintillation counter (Gamma 5500, Beckman Instruments, Inc., Schaumburg, IL). Each tube is counted for one full minute. After counting, the tubes must be disposed of in the appropriate drums.

10. After examining the reports, any duplicates which show more than a 25% difference between samples should be re-analyzed.

Testosterone Radioimmunoassay Procedure

1. Label four plain 12 x 75 mm polypropylene test tubes T (for total counts) and NSB (nonspecific binding) in duplicate.

2. Label 12 testosterone antibody-coated tubes (Diagnostic Products Corp., Los Angeles, CA) A-F in duplicate for calibrators. Label antibody-coated tubes for subject samples starting with number 17 in duplicate. Label key to associate subject samples with respective tube number.

3. Pipette 50 μl of the A calibrator in duplicate into the NSB and A tubes. Pipette 50 μl of each B-F calibrator in duplicate into its respective tube. All samples should be pipetted directly to the bottom of the tube.

4. Pipette 50 μl of each subject sample, thawed at room temperature, in duplicate into its appropriate tube. All samples should be pipetted directly to the bottom of the tube. Change pipette tips following the duplicate samples. Use gloves and follow universal precautions against blood-borne diseases.

5. Add 1.0 ml of ^{125}I Testosterone to every tube using a measuring pipette designated for isotope use. Dispensing of the tracer should take no more than 10 minutes. Gently vortex each tube. Survey gloved hands and working area with Geiger counter.

6. The T-tubes should be set aside under the hood since they require no further processing until counting.

7. Cover the rack of tubes with aluminum foil. Place the tubes in a water bath heated to 37^{o} C. Incubate for three hours.

8. After 45 minutes, remove the tubes and take back to the hood to decant. Fill the pans labeled “Waste” and “Rinse” about 25% full with water and place in hood. Place a mesh rack over the test tube rack, keeping hands at the ends. Gently invert.the rack to drain into the waste pan. Next, place the rack into the rinse pan. Tap out any excess water. Turn over the rack and leave in hood on tray covered with absorbent paper. Gently slide of the mesh rack. The tubes may be left in the hood to air dry before counting. All radioactive waste is placed into the large waste container in the hood. The waste pan can be rinsed in the hood. Both pans can then be decontaminated and cleaned in a sink designated for cleaning. Survey hands and all areas.

9. Place all tubes (T tubes included) in numerical order in the scintillation counter (Gamma 5500, Beckman Instruments, Inc., Schaumburg, IL). Each tube is counted for one full minute. After counting, the tubes must be disposed of in the appropriate drums.

10. After examining the reports, any duplicates which show more than a 25% difference between samples should be re-analyzed.

Insulin Radioimmunoassay Procedure

1. Label four plain 12 x 75 mm polypropylene test tubes T (for total counts) and NSB (nonspecific binding) in duplicate.

2. Label 14 insulin antibody-coated tubes (Diagnostic Products Corp., Los Angeles, CA) A-G in duplicate for calibrators. Label antibody-coated tubes for subject samples starting with number 19 in duplicate. Label key to associate subject samples with respective tube number.

3. Pipette 200 μl of the A calibrator in duplicate into the NSB and A tubes. Pipette 200 μl of each B-G calibrator in duplicate into its respective tube. All samples should be pipetted directly to the bottom of the tube.

4. Pipette 200 μl of each subject sample, thawed at room temperature, in duplicate into its appropriate tube. All samples should be pipetted directly to the bottom of the tube. Change pipette tips following the duplicate samples. Use gloves and follow universal precautions against blood-borne diseases.

5. Add 1.0 ml of ^{125}I Insulin to every tube using a measuring pipette designated for isotope use. Dispensing of the tracer should take place no more than 40 minutes following pipetting of first sample. Gently vortex each tube. Survey gloved hands and working area with Geiger counter.

6. The T-tubes should be set aside under the hood since they require no further processing until counting.

7. Cover the rack of tubes with aluminum foil. Place the tubes on a tray covered with plastic-backed absorbent paper. Incubate in the hood for 18-24 hours at room temperature.

8. After 24 hours, the tubes are ready to decant. Fill the pans labeled "Waste" and "Rinse" about 25% full with water and place in hood. Place a mesh rack over the test tube rack, keeping hands at the ends. Gently invert the rack to drain into the waste pan. Next, place the rack into the rinse pan. Tap out any excess water. Turn over the rack and leave in hood on tray covered with absorbent paper. Gently slide of the mesh rack. The tubes may be left in the hood to air dry before counting. All radioactive waste is placed into the large waste container in the hood. The waste pan can be rinsed in the hood. Both pans can then be decontaminated and cleaned in a sink designated for cleaning. Survey hands and all areas.

9. Place all tubes (T tubes included) in numerical order in the scintillation counter (Gamma 5500, Beckman Instruments, Inc., Schaumburg, IL). Each tube is counted for one full minute. After counting, the tubes must be disposed of in the appropriate drums.

10. After examining the reports, any duplicates which show more than a 25% difference between samples should be re-analyzed.

Growth Hormone Radioimmunoassay Procedure

1. Label 18 plain 12 x 75 mm polypropylene test tubes in duplicate T (for total counts), NSB (nonspecific binding), and A-G for calibrators (Diagnostic Products Corp., Los Angeles, CA).

2. Label plain tubes for subject samples starting with number 19 in duplicate. Label key to associate subject samples with respective tube number.

3. Pipette 100 μl of the A calibrator in duplicate into the NSB and A tubes. Pipette 100 μl of each B-G calibrator in duplicate into its respective tube. All samples should be pipetted directly to the bottom of the tube.

4. Pipette 100 μl of each subject sample, thawed at room temperature, in duplicate into its appropriate tube. All samples should be pipetted directly to the bottom of the tube. Change pipette tips following the duplicate samples. Use gloves and follow universal precautions against blood-borne diseases.

5. The T-tubes should be set aside under the hood since they require no further processing until counting.

6. Add 100 μl of the blue antiserum to all tubes except T and NSB. Vortex gently.

7. Cover rack with foil and incubate for one hour at room temperature.

8. Add of 100 μl ^{125}I Human Growth Hormone (yellow-colored) to every tube using a measuring pipette designated for isotope use. Gently vortex each tube. All tubes should appear yellow. Survey gloved hands and working area with Geiger counter.

9. Cover rack with foil and incubate for one hour at room temperature.

10. Add 1.0 ml well-mixed, cold Precipitating Solution (blue) to all tubes. Vortex gently. All tubes should appear blue.

11. Centrifuge for 15 minutes at 3,000 x g.

12. After 15 minutes, remove the tubes and take back to the hood to decant. Fill the pans labeled “Waste” and “Rinse” about 25% full with water and place in hood. Place a mesh rack over the test tube rack, keeping hands at the ends. Gently invert the rack to drain into the waste pan. Next, place the rack into the rinse pan. Tap out any excess water. Turn over the rack and leave in hood on tray covered with absorbent paper. Gently slide of the mesh rack. The tubes may be left in the hood to air dry before counting. All radioactive waste is placed into the large waste container in the hood. The waste pan can be rinsed in the hood. Both pans can then be decontaminated and cleaned in a sink designated for cleaning. Survey hands and all areas.

9. Place all tubes (T tubes included) in numerical order in the scintillation counter (Gamma 5500, Beckman Instruments, Inc., Schaumburg, IL). Each tube is counted for one full minute. After counting, the tubes must be disposed of in the appropriate drums.

10. After examining the reports, any duplicates which show more than a 25% difference between samples should be re-analyzed.

<u>Glucose Colormetric Assay</u> (Kit #555, Sigma Chemical Co., St. Louis, MO)

1. Prior to starting the assay, prepare 0.1 N hydrochloric acid (HCl) solution by diluting 8.6 ml of concentrated HCl to 1,000 ml in a volumetric flask. Use distilled water for the dilution. Work under the hood, and wear goggles, gloves, and a protective lab coat while preparing the solution. The solution can be poured into an automatic pipetter (Repipet, Labindustries, Berkeley, CA) with the remaining solution poured into an appropriately labeled storage bottle. Carefully rinse all glassware.

2. Turn on spectrophotometer and set wavelength to 520 nm. The spectrophotometer should warm up at least 30 minutes.

3. Prepare the glucose assay reagent by adding 17 ml distilled water to 1 vial of glucose color reagent. The solution should be mixed by gentle inversion.

4. For the assay, 16 x 125 mm test tubes (14-958Q, Fisher Scientific, Pittsburgh, PA) can be used. One tube can be used for the blank, but duplicates should be labeled for the standards and subject samples. Since the samples should be read on the spectrophotometer within 30 minutes following the addition of the acid solution, an appropriate amount of subject samples should be thawed to assay in this time period. New blanks and standards are required with every batch of subject samples.

5. The blank tube receives 20 μl distilled water and 1.0 ml glucose assay reagent.

6. The standard tubes receive 20 μl glucose standard (5.56 mmol/l) and 1.0 ml glucose assay reagent.

7. The subject sample tubes receive 20 μl serum and 1.0 ml glucose assay reagent.

8. All tubes are mixed by gentle swirling, and the tubes should stand for 5-10 minutes at room temperature. The solutions in the tubes will begin to turn a reddish-brown color.

9. All tubes receive 10 ml of the 0.1 N HCl solution using the automatic pipette dispenser. Vortex each tube to mix.

10. The solutions should be transferred to 10 x 10 x 45 mm cuvettes (#67.741, Starstedt, Newton, NC) and read at 520 nm. The blank should be read first, and the spectrophotometer zeroed. The standards and subject samples are read next. All readings should be completed within 30 minutes.

11. Duplicates which show differences of more than 10% should be re-analyzed.

12. Serum glucose (mmol/l) is calculated by:

$[Absorbance_{sample}/Absorbance_{standard}] \times 5.56$

13. The assay obeys Beer's law up to a glucose concentration of 16.67 mmol/l. Samples which read higher should be diluted with an equal volume of the 0.1 N HCl solution, remeasured on the spectrophotometer, and multiplied by 2.

Creatine Kinase (CK) Enzymatic Assay (Kit # 47, Sigma Chemical Co., St. Louis, MO)

1. Turn on spectrophotometer and set wavelength to 340 nm. The spectrophotometer should warm up at least 30 minutes. A constant temperature of 30^{o} C in the cuvette chamber is recommended although temperatures of 25^{o} C and 37^{o} C can also be used with modifications to the calculations. Chamber temperature can easily be maintained by running a heated water bath through the chamber.

2. The subject samples should be thawed at room temperature just prior to their use in the assay. Control serum (Accutrol, Sigma Chemical Co.) is reconstituted by adding 5.0 ml distilled water to the vial.

3. The CK assay reagent is reconstituted by adding 50 ml distilled water to the vial. The vial should be mixed by gentle inversion. The reagent needs to be at reaction temperature before it can be read in the spectrophotometer. This can be accomplished by heating test tubes of reagent to 30^{o} C in a heating block. The reagent is stable for 4-8 hours at this temperature (personal communication, Sigma Chemical Co.).

4. All controls and subject samples should be assayed in duplicate. For control and subject samples, 20 μl of the serum and 1.0 ml of the heated CK reagent are added

to a 10 x 4 x 45 mm cuvette (#67.642, Starstedt, Newton, NC). The cuvette is covered with laboratory film and mixed by inversion.

5. The cuvette is placed in the heated chamber and incubated for three minutes.

6. At the end of the three minutes, absorbance is recorded as Initial Absorbance (A). Absorbance is monitored at 30 sec intervals to check for linearity in the assay. Final A is recorded at 120 seconds (2 minutes).

7. Change in absorbance per minute (ΔA/min) is calculated by subtracting Initial A from Final A and dividing by two.

8. Serum CK(U/l) is calculated by ΔA/min x 8,200. If duplicate samples show greater than 10% difference, a triplicate sample should be assayed. The mean serum CK for Accutrol control samples is 130 U/l with a range of 100-160 U/L.

9. Samples should be diluted with equal volume of 0.85% normal saline (0.85 g sodium chloride diluted to 100 ml) if the ΔA/min is greater than 0.250. Results should be multiplied by 2 to compensate for the dilution.

Glycogen Colormetric Assay (Lo, Russell, & Taylor, 1970)

1. Prepare glycogen standards by weighing 10 mg of rabbit liver glycogen standard (G8876, Sigma Chemical Co.) on an analytical balance and diluting to 10 ml with distilled water. Vortex well to mix. The concentration of this solution is 1,000 μg/ml. From this solution take 1.0 ml and dilute again to 10 ml with distilled water to make a glycogen standard of 100 μg/ml. For these dilutions an electronic balance can be used. Continue dilutions of the 100 μg/ml solution to make standards of 5, 25, 50, and 75 μg/ml. The standards remain stable for only about two days. Therefore, new standards must be frequently prepared.

2. Prepare 30% potassium hydroxide (KOH) solution by dissolving 30 g of KOH pellets to 100 ml distilled water in a volumetric flask. Work under the hood using gloves and goggles. This solution becomes very hot so it must be prepared over ice. Once the solution cools, transfer it to a beaker, and stir in sodium sulfate (Na_2SO_4) until the Na_2SO_4 no longer goes into solution and forms a precipitate.

3. Prepare 5% phenol solution by dissolving 25 g phenol crystals to 500 ml of distilled water using a volumetric flask. Work under the hood using gloves and goggles.

4. Prepare boiling water bath under the hood before beginning the assay.

5. Label polypropylene tubes for each muscle sample that will be assayed.

6. Take samples from -80^{o} C freezer on ice and place immediately into polypropylene test tube. Work under the hood while handling all chemicals and use protective goggles and gloves.

7. Add 0.5 ml of the 30% KOH solution and vortex well. Process samples as quickly as possible after taking out of cryovial to avoid degradation of glycogen.

8. Cap tubes with polypropylene caps with holes punched to allow venting. Place in boiling water bath for 30 minutes.

9. Cool the tubes for about 5 minutes over ice.

10. Add 5.0 ml 95% ethanol solution to each tube and vortex well.

11. Allow the tubes to sit on ice for 30 minutes, or refrigerate the samples overnight to precipitate glycogen.

12. Centrifuge the tubes for 30 minutes at 1000 x g to produce glycogen pellet.

13. Carefully aspirate supernatant with transfer pipette so as not to disturb pellet.

14. Dissolve pellet in 3.0 ml distilled water and vortex well.

15. Using glass disposable 16 x 125 mm test tubes (#14-958Q, Fisher Scientific, Pittsburgh, PA) pipette 1.0 ml water into tube labeled blank, pipette 1.0 ml each standard in duplicate, and pipette 1.0 ml samples in duplicate.

16. Add 1.0 ml 5% phenol to all tubes.

17. Rapidly add 5.0 ml concentrated sulfuric acid (H_2SO_4) to all tubes using an automatic pipette dispenser. Direct the stream of the acid into the liquid and not on the sides of the test tube to facilitate mixing. The test tubes will become hot and must cool for 30 minutes.

18. Turn on spectrophotometer, set the wavelength to 490 nm, and let the spectrophotometer warm up for 30 minutes.

19. After the tubes have cooled, pour into 10 x 10 x 45 mm cuvettes (#67.741, Starstedt, Newton, NC), measure and record absorbances of all standards and subject samples versus blank. If a sample shows greater absorbance than the 100 μg/ml standard, dilute the sample appropriately with blank and remeasure. Multiply by the appropriate dilution during calculations.

20. Obtain standard curve by taking average absorbances of the standards and performing a regression analysis using a spreadsheet computer application. The slope of the curve is needed for calculations.

21. Glycogen (g glycogen/100 g tissue) is calculated by:
(Average absorbance of sample at 490 nm/slope of standard curve)
x (Total Volume of glycogen solution [3.0 ml])/volume of aliquot used in reaction)

x (10^{-4}/weight of muscle sample in g)
x Dilution factor (if any).

22. To obtain glycogen content in mmol/kg ww, divide the glycogen concentration obtained in g/100 g by 162 (molecular weight of glycogen).

Urinary 3-Methylhistidine (Bidlingmeyer, Cohen, & Tarvin, 1984)

1. The urine samples are thawed at room temperature.

2. A 1.0 ml aliquot of each sample is mixed 1:1 with a methionine sulfone (MetSo) internal standard. This mixture is manually filtered (PLGC Ultrafitratration Membrane, Millipore Corp., Milford, MA) to exclude proteins and peptides that have a molecular weight greater than 10,000 Daltons. The samples may be either frozen and stored at this time or used in analysis.

3. For analysis, 25 μl of each filtered sample is pipetted in duplicate into 6 x 50 mm test tubes. A Hamilton syringe should be used.

4. Using forceps, the tubes are placed into a large vacuum vial that can hold 12 tubes. After the cap is placed on, the tubes are placed in a vacuum station and dried to 70 mTorr. This drying step removes solvents and volatile components such as HCl. After they are dried, the tubes may be stored in a dessicator jar for up to two weeks in the freezer.

5. To begin the derivitazation process, 10 μl of redry solution (2:2:1 ratio of HPLC grade methanol, 1M sodium acetate, and triethylamine [TEA]) are added to each sample and standard, and all tubes are vortexed. All samples are dried to 50 mTorr for 15 minutes. This step is repeated.

6. Freshly prepared derivatization reagent (7:1:1:1 ratio of HPLC grade methanol, TEA, DiH_20, and Phenylisothiocyanate [PITC]) is added (20 μl) to each tube. All tubes are vortexed and placed in the vacuum station (without vacuum on) to sit for 10 minutes. Then the samples are dried to 50 mTorr and remain at 50 mTorr for 30 minutes. The PITC reacts with the primary and secondary amino acids to form phenylthiocarbamyl derivatives (PTC).

7. To each tube add 100 μl diluent (#88119, Waters Association, Milford, MA) and vortex. The tubes should be placed in a microcentrifuge for 30 seconds at 1,200 rpm.

8. The supernatant is aspirated from each sample and placed into a WISP vial. The samples should be run as soon as possible but may be stored up to 24 hours in a refrigerator.

9. The WISP vials are placed on the high performance liquid chromatography system (HPLC) (Pico·Tag Amino Acid Analysis Sytem, Waters Division of Millipore Corp., Milford, MA). The PTC derivatives are separated by reverse phase chromatography. A silica-based chromatography column specifically for free amino acids is used (#10950, Waters Division). Elucidation of 3MH takes approximately 30 minutes. Standards are placed at multiple points during each run.

10. Concentration of 3MH in an unknown (A) is determine by the formula:
(B * C)/D * E * F
B = Concentration of 3MH in standard
C = Peak area of 3MH in the unknown
D = Peak area of 3MH in the standard
E = Dilution factor (in this assay, 4)
F = Peak area of internal standard in standard / peak area of internal standard in unknown

Original output is in μmoles/deciliter. Output is transformed to mg/l and can be multiplied by the urine volume in liters.

Creatinine Colormetric Assay (Kit #555, Sigma Chemical Co., St. Louis, MO)

1. Prepare alkaline picrate solution by combining 50 ml 1.0 N sodium hydroxide (NaOH) from the kit into 250 ml of the Creatinine Color Reagent (also provided in the kit). Work under the hood. In addition, the color reagent contains picric acid that can be explosive when dry.

2. Unthaw urine samples at room temperature. Urine must be diluted at least by a factor of 10 for the assay. Dilute with distilled water in test tubes.

3. Turn on spectrophotometer, set the wavelength to 500 nm, and let the spectrophotometer warm up for at least 30 minutes.

4. For each set of assays, it is necessary to run one blank plus duplicate standards and controls. Use cuvettes capable of 4 ml reaction volume (size 10 x 10 x 45 mm, #67.741, Starstedt, Newton, NC). Pipette 300 μl distilled water in cuvette for the blank. Pipette 300 μl of the creatinine standards, controls, and diluted subject samples in duplicate into appropriate cuvettes.

5. Add 3.0 ml alkaline picrate solution to all cuvettes and mix well. Allow the cuvettes to stand 8-12 min at room temperature under the hood.

6. Determine Initial Absorbance (A) of standards, controls, and samples versus the blank. Record all Initial A.

7. Add 100 μl acid reagent (from the kit) to all cuvettes and immediately mix. Allow the cuvettes to stand for 5 minutes at room temperature.

8. Determine Final A of standards, controls, and samples versus blank. Record all Final A.

9. Determine creatinine concentration (μmol/l) for subject samples and controls by: (Initial $A_{\text{control or sample}}$ - Final $A_{\text{control or sample}}$) / (Initial A_{standard} - Final A_{standard}) x Standard Concentration x 88.4

10. To obtain creatinine concentration (mmol/24 hr): ([Creatinine concentration (μmol/l) x Urine volume in liters] /1000) x dilution factor.

11. If duplicate samples show greater than 10% difference, a triplicate sample should be assayed.

Statistics

Descriptive statistics for subject characteristics were analyzed using Microsoft Excel Version 5.0 for Windows (Microsoft Corp., Redmond, WA). A one-way analysis of variance (Microsoft Excel 5.0) revealed no differences between treatment groups in baseline subject characteristics of age, height, weight, 1-RM, and VO_{2peak}. Statistical significance was set *a priori* at $P < 0.05$.

All blood, urine, exercise performance, and muscle soreness data were analyzed using a repeated measures analysis of variance (ANOVA) to assess group by time interactions (Statistical Analysis Systems [SAS], Version 6.12 for Windows, Cary, NC). The data for morning CK underwent a logarithmic transformation to normalize the data. Muscle soreness and blood parameters such as serum CK, testosterone, cortisol, growth hormone, insulin, and glucose underwent separate repeated measures analyses for the morning values and the values on the day of eccentric exercise. Repeated measures analysis of covariance was also performed on body weight with body weight on day 1 as the covariate and on day 5 serum CK with the fasted morning CK value as the covariate. One-way ANOVA was used to examine daily energy and macronutrient data. Student

Newman-Keuls post-hoc analyses were employed in the presence of significant F-ratios. When the post-hoc analyses from repeated measures ANOVA on urinary 3MH revealed significant differences between groups on day 5, a separate one-way ANOVA was performed for 3MH on this day only. Results of statistical analyses on dependent variables can be found in Appendix N. All p-values from repeated measures ANOVA are reported adjusted for the Hunyh-Feldt epsilon. Raw data are reported as means ± SE (Appendix O).

Appendix B

Human Subjects Committee Request

REQUEST FOR APPROVAL FOR RESEARCH PROPOSAL
IN THE DEPARTMENT OF HUMAN NUTRITION AND FOODS

INVESTIGATORS:

Janet Walberg Rankin, Ph.D. (Associate Professor in Human Nutrition and Foods)
Janet R. Wojcik, M.S. (doctoral candidate in Human Nutrition and Foods)

TITLE: Effect of Milk Beverage on Glycogen Resynthesis and Extent of Muscle Damage Induced by Resistance Exercise

BACKGROUND AND JUSTIFICATION:

Although much of the focus of the nutrition and athletic performance literature has been on nutrient consumption prior to and during exercise, nutrition following exercise may also be critical for some athletes. Some athletes participate in multiple workouts per day (e.g. triathletes or cross trainers) or multiple competitions in one day (e.g. swimmers) or consecutive days (e.g. wrestlers, runners). These athletes would like to enhance recovery of metabolic deviations which occur as a result of intense exercise, e.g. muscle glycogen replacement, repair of damaged muscle tissue. This proposal will review the evidence for an effect of resistance exercise on muscle carbohydrate and protein breakdown and pursue the hypothesis that consumption of a milk beverage could enhance recovery of these metabolic disruptions.

Acute Effects of Eccentric Resistance Exercise. Dynamic muscular contraction can be concentric where the muscle produces force while shortening, or eccentric where the muscle produces force while lengthening. Eccentric contractions induce the most muscle damage and protein breakdown (Evans & Cannon, 1991). Although research has focused on aerobic exercise with eccentric contractions (e.g. downhill running), it has been shown resistance exercise also induces muscle damage and protein breakdown (Evans et al., 1986; Frontera et al., 1988; Hickson & Hinkelmann, 1985; Pivarnik et al., 1989).

Although there is evidence of acute mechanical damage within the muscle occurring with eccentric exercise, a progression of metabolic changes can be shown after exercise in order to clear and repair the damaged area. Two of the most widely studied metabolic markers of muscle damage are plasma creatine kinase (CK) and urinary 3 methylhistidine (3MH). CK is rapidly elevated by 24 h post-exercise while urinary 3MH excretion is typically increased several days following acute exercise (Evans et al., 1986). Other indicators of skeletal muscle protein breakdown include cytokines Interleukin-1β (IL-1), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). High levels of IL-1 are believed to be involved in increased muscle protein breakdown and may have a more immediate effect than IL-6 (Evans, 1991). TNF-α increases production of oxygen free radicals in the cell and can also mediate an inflammatory response (Evans & Cannon, 1991).

Methods to Attenuate Post-Exercise Protein Breakdown and Inflammation. It is possible that macronutrient intake could affect damage via provision of substrate or change in hormonal environment. Cade et al. (1991) tested the effect of several different beverages on blood CK and lactate dehydrogenase (LDH), indicators of muscle cell damage, in intercollegiate swimmers. They reported that a "milk protein supplement" (250-500 ml containing 15 g of lactalbumin and 16% sucrose) consumed just after the swim workout was superior to a carbohydrate/electrolyte drink with regard to the CK and LDH response. A follow up study looking at the time course over 24 hr after the exercise of the blood enzyme reduction to baseline levels following an intense swim workout showed that the return of LDH to baseline levels was more rapid when the swimmers ingested the milk/sucrose supplement than when they only ingested water or a carbohydrate solution. They hypothesized that the milk/sucrose beverage was providing essential amino acids necessary for resynthesizing catabolized proteins and allowed more rapid recovery of the contractile apparatus. Results of this study are provocative but because of a variety of design flaws (e.g. volume of milk/sucrose beverage was not controlled and varied between 250 and 500 ml, lack of random assignment of order of dietary treatments) more research is necessary to determine whether dietary carbohydrate and/or protein intake can influence muscle damage and protein catabolism.

Chandler et al. (1994) studied whether varying dietary consumption following resistance exercise influenced hormones known to affect protein synthesis: growth hormone, insulin, and testosterone. Subjects consumed either water or an isocaloric carbohydrate (1.5 g/kg, mix of dextrose and maltodextrose), protein (1.38 g/kg, milk protein isolate and whey protein isolate), or a mix of protein and carbohydrate (1.06 g/kg carbohydrate and 0.41 g/kg protein) immediately and 2 h after the resistance exercise. They reported that the insulin response was highest for the combination beverage immediately post exercise and was significantly different from all treatments except the carbohydrate trial. The insulin value at 8 h post exercise was significantly higher for the combination beverage as compared to all other treatments. The growth hormone response to the carbohydrate and protein mix was elevated relative to the protein supplement and water trials at 6 h post exercise. They interpreted this as a more favorable environment for protein anabolism after exercise in the carbohydrate trial and particularly the carbohydrate with protein mix. No actual measurements of protein status were done. This study suggests, however, that a combination of protein and carbohydrate consumed after resistance exercise could increase the hormones which stimulate protein synthesis (testosterone, growth hormone, insulin) and those that reduce protein catabolism (insulin).

Glycogen Breakdown and Resynthesis after Resistance Exercise. Typically, athletes are recommended to consume a carbohydrate beverage following exercise to enhance recovery of muscle glycogen stores that are utilized during exercise. Most of this research has been done following aerobic exercise. One group has shown that glycogen replacement post aerobic exercise was actually faster with a carbohydrate/protein beverage compared to a carbohydrate beverage alone (Zawadzki et al., 1992). They attribute the faster rate of glycogen synthesis to an elevated insulin response to the carbohydrate/protein combination. However, this research is difficult to interpret since the dietary treatments were not isocaloric (i.e. carbohydrate = 448 kcal, protein = 163 kcal, carbohydrate + protein = 611 kcal). In addition, the absolute muscle glycogen content was not different between treatments at the 4 h measurement. Only the change from baseline to 4 h was significantly different between

treatments. Since the carbohydrate/protein group started with a lower muscle glycogen this may have influenced the results. This work should be extended to test isocaloric treatments in groups beginning with similar muscle glycogen concentrations.

Several studies have looked at glycogen depletion and repletion with resistance exercise. Muscle glycogen utilization during exercise is typically more rapid than with aerobic exercise but since the events are shorter the magnitude of depletion is modest. This reduction may still be critical for performance since the depletion is likely to be in specific motor units, muscle fibers, and compartments within the cell critical for high intensity work (Friden et al., 1989).

The type of muscle contraction during resistance exercise has been shown to affect rate of glycogen replacement Doyle et al. (1993) had subjects do resistance exercise (10 sets of 10 repetitions) following a prolonged cycling bout. One leg did the contractions eccentrically while the other did only concentric contractions. Glycogen replacement was similar for each leg for the first 4 h following the exercise but was 25% less on the third day after a second cycling bout in the muscle that had done eccentric exercise compared to that which did concentric exercise.

One study investigated the effect of a dietary intervention on the rate of glycogen replacement after resistance exercise. Pascoe et al. (1993) confirmed that consumption of a carbohydrate beverage (1.5 g/kg immediately and 1 h after exercise) following resistance training enhanced the rate of glycogen replacement relative to water. The effect of a combination of carbohydrate with protein on muscle glycogen replacement after resistance exercise has not been examined. This combination may be superior to carbohydrate alone since, as described above, Zawadzki et al. (1992) found this to be the case following aerobic exercise.

PROCEDURES:

Subjects. Twenty-seven males will be recruited via posted fliers in the Virginia Tech gymnasium and student center. These individuals will be between 18 and 30 years of age, not involved in any resistance (weight training) exercise for at least 1 year, and be free of any cardiovascular, renal, orthopedic, or metabolic diseases such as diabetes which would preclude their involvement in strenuous exercise or affect their metabolic responses to exercise. A copy of the screening form is attached. After being informed of the procedures and risks, those who remain will sign the informed consent form. They will be asked to agree to only eat the food provided by the experimenters, do no other exercise, and refrain from taking either nonsteroidal anti-inflammatory medications or vitamin supplements during the study period.

Design. The study period will consist of a total of 9 d with the first 2 d being a baseline period, resistance exercise bout on d 3, and continued measurements for 6 more days. The start date of the experiment will be staggered such that 6-7 subjects (with random assignment to the groups) will begin on each of 4 consecutive days. This will allow the testing on d 3 to occur for all subjects within a 8 - 10 am time frame. All subjects will be provided with all food throughout the 9 d. All will undergo the same measurements on d 3 but will be randomly assigned to consume one of 3 beverages after the exercise bout. Data collection will include daily total urine collections, daily morning fasted blood withdrawal on

d 3-9 with additional blood collections on d 3 during acute recovery from the exercise bout (immediate post, 3 and 6 h post). Muscle biopsies will be taken from the vastus lateralis immediately after the resistance exercise (d 3), and at 24 (d 4) and 72 h (d 6) after the exercise.

Dietary Control. Subjects will be fed a meat free, minimal dairy foods (2 servings per day) diet on a 3 d menu rotation for the 9 d of the study at Wallace Annex, a metabolic feeding unit on campus operated by the Department of Human Nutrition and Foods. This controlled feeding will be under the direction of Ms. Christina Baum, who previously directed a 13-wk controlled feeding study at Wallace Annex for her master's thesis in the Department of Human Nutrition and Foods. To ensure hygiene and safety, any personnel who handle food will wash their hands prior to handling or serving food, and long hair will be tied back away from food.

All subjects will eat breakfast and dinner in the facility and be given a bag lunch. Any bag lunch which requires refrigeration will be given in a cooler assigned to the subject for the duration of the study. The diet will be designed at 50 kcal/kg (Pivarnik et al., 1989) with 55% carbohydrate, 30% fat, and 15% protein (primarily egg and vegetable sources). Energy intake will be varied as necessary to maintain body weight. Breakfast will not be fed on d 3 since the subjects will consume their treatment beverage following the morning exercise bout. The control group (consumes no energy during this period) will be given a larger lunch and an afternoon snack on that day to balance the energy fed to the other two groups in the morning. On days 4-9, all subjects will consume a similar diet.

The subjects will receive one of three dietary treatments following the resistance exercise protocol on d 3: water, carbohydrate, or milk beverage. One bolus will be fed immediately after and at 2 hours after the exercise. Each "milk" and carbohydrate treatments will be isocaloric at 5 kcal/kg resulting in a total consumption of 10 kcal/kg over the 2 hours. The carbohydrate beverage will be Exceed High Carbohydrate (Weider) which contains hydrolyzed cornstarch, glucose, and sucrose as the carbohydrate sources. The "milk" beverage will consist of nonfat milk mixed with chocolate flavoring (Nestle Quick, at 22 g per 8 oz. milk). This will result in a beverage containing 73% carbohydrate (primarily sucrose and lactose), 23% protein, and 3% fat. For example, an 80 kg individual will consume 400 kcal immediately after and another 400 kcal 2 h after the eccentric exercise bout. These post exercise feedings will replace the breakfast for the morning on d 3. The diet for the remainder of d 3 will continue as on the other days.

VO2max test. Two weeks prior to the experimental period, groups of 4-5 subjects will report to the lab following an overnight fast. Following a light warm-up period, these subjects will undergo an incremental cycling protocol to volitional exhaustion performed on a Monarch stationary cycle ergometer. Initial workload will be set at 25 Watts (W) which equates to a friction belt resistance of 0.5 kg and pedaling frequency of 50 RPM. Workload will be increased by 25 W (0.5 kg additional resistance on the friction belt) every 2 min until pedaling frequency can no longer be maintained. Heart rate will be monitored by ECG telemetry. Expired oxygen (O2) and carbon dioxide (CO2) will be analyzed using a Med Graphics CPX metabolic cart (Medical Graphics Corp., St. Paul, MN). This requires the subject to breathe into a mouthpiece throughout the test. The nose is pinched with a noseclip similar to those used by swimmers. Lightweight plastic tubing connects the mouthpiece to the metabolic cart. Maximal O2 uptake (VO2max) will be determined if a leveling-off of O2

consumption is seen despite increased workload, or peak O2 uptake (VO2peak) will be determined as the highest O2 consumption measured from expired gas analysis.

Performance test One week prior to the experimental period, subjects will undergo testing for peak quadriceps (thigh) torque during concentric leg extension, a shortening muscular contraction of the thigh. This test will be performed on a Biodex System II isokinetic dynamometer (Biodex Corp., Shirley, NY). The Biodex looks similar to a Nautilus or Universal leg extension weight lifting station. This type of machine is widely used in sports settings because it can be used to test for muscle strength, to train for muscular fitness improvements, and to rehabilitate injuries. The subject will sit down in a chair attached to the Biodex machine, and his dominant leg will be strapped down and held in two places: over the thigh on the seat of the chair and into a pad which covers the shin. This shin pad is attached to an arm which connects to the measurement device in the machine. The speed of the machine can be controlled by the experimenter. This machine matches the resistance produced by the subject (accommodating resistance). The subject will perform 5 concentric (muscle shortening) leg extensions at a speed 30 degrees per second. The highest torque value will be considered peak torque, or measure of maximal muscle force. In addition, the average work per repetition will be calculated. Subjects will be manually assisted on leg flexion (back to the starting position) in order to avoid performing eccentric (lengthening) muscular contractions. This performance test will be repeated on d 4, 5, and 6 of the study in order to examine effects of the eccentric exercise bout and of the interventions on peak and average torque. This type of testing is very safe since the machine accommodates to the force generated by the subject. If the subject reduces their force production, the machine automatically reduces the resistance. The peak muscular force will be determined following this brief test. You will repeat this test on days 4, 5, and 6 of the study. We ask that you perform no other exercise, aerobic or strength, outside what is part of the study.

Aerobic glycogen reduction exercise. On d 3 all subjects will cycle for 45 min at 70% of their peak VO2 on a Monark cycle ergometer with the goal of further reducing muscle glycogen.

Eccentric exercise protocol to induce muscle damage. Thirty minutes following the aerobic glycogen-reducing exercise on d 3, subjects will perform a resistance exercise protocol. This protocol, a modification of that used by Doyle et al. (1993) will require subjects to perform repeated sets of eccentric leg flexions (thigh muscle lengthening contractions) at 30 degrees/second on the Biodex System II. The workload will be set at 120% of the concentric peak torque determined during the first performance test. Total work performed will be the endpoint and will be set at 10 times the work done during the concentric test. Thus, all subjects will perform similar amounts of work relative to their maximal concentric effort. The time for the eccentric contraction will be controlled at 4 seconds. Subjects will be manually assisted on leg extension in order to avoid performing concentric muscular contractions of the quadriceps. Rest between sets will be controlled at 60 s.

Blood. Blood samples (12 ml each) will be withdrawn daily by Ms. Janet Rinehart, medical laboratory technician in Human Nutrition and Foods, after an overnight fast on d 3-9 as well as immediately and 3 and 6 h after the eccentric exercise bout on d 3. Subjects will be asked to remain in the laboratory for 20 min following the blood sampling. All blood samples will be analyzed for total CK, IL-1, and TNF. The blood samples taken on d 3 will

also be analyzed for glucose as well as the hormones insulin, testosterone, growth hormone, and cortisol.

Urine. Subjects will be given polypropylene jugs with hydrochloric acid as a preservative each day of the study to be used to collect 24 h urine samples. Each 24 h sample will begin with the second urination of that day. The collections will be brought to the laboratory daily for volume measurement and freezing of an aliquot for later analysis of 3-methylhistidine, an indicator of skeletal muscle protein breakdown, and creatinine by automated amino acid analyzer.

Muscle biopsy and analysis. A 25-75 mg portion of the vastus lateralis muscle of the eccentrically exercised leg will be removed using the needle biopsy technique with suction immediately after the eccentric exercise bout, 24 and 72 h after the bout. This procedure will be performed by Ms. Janet Rinehart, and she will be observed by a physician. The subject will be asked to lie on a flat cot. The hair on the leg will be shaved and cleaned with iodine (will make the skin temporarily yellow). A sterile drape will be placed over the skin site. A substance used to cool the skin (ethyl chloride) will be sprayed on the skin in order to numb the area. A local anesthetic, xylocaine, will be injected over the site in several punctures. This procedure may sting, similar to a bee sting. After the area is numb, a small incision about 1/2 inch wide will be made in the skin with a scalpel blade. Pressure will be applied with a sterile gauze pad to reduce bleeding. Then the sterile biopsy needle about the diameter of a pencil (1/4 inch) will be inserted about 1 inch into the thigh. A piece of muscle about half the size of a pencil eraser (50-100 mg) will be removed. The time required to insert the needle and remove the sample is about 3-5 seconds. Following removal of the needle, pressure will be applied to the area for 20 minutes to reduce bleeding and swelling. The incision will be closed with a sterile strip and a Band-Aid; it does not require stitches. Subjects will be left with a small scar, which will fade with time, as a result of the incision.

Following these second and third samples, subjects will be asked to remain in the laboratory for at least 20 minutes so they can be monitored. They will be asked to keep the pressure wrap on the leg for 8 hours after the biopsy, and the steristrip and Band-Aid on for 3 days. Subjects will be instructed to keep the site clean, as with any cut, to reduce risk of infection. Following the last biopsy, subjects will be asked to return to the lab 3 days later so the site can be examined for appropriate healing. The 2nd and 3rd biopsies will be taken at least 3 cm distal to the previous incision since the incision has been shown to delay glycogen resynthesis (Costill et al., 1990). The sample will be frozen in isopentane cooled with liquid nitrogen within 30 s, stored at -80 degrees, and enzymatically analyzed later for glycogen.

Muscle Soreness. Soreness of the front thigh muscles will be assessed using a 10 point scale (1= no soreness, 10= unbearable soreness) on the morning of d 4-9 prior to the blood withdrawal. The scale will be explained to the subjects who will subjectively assign a number which represents their soreness perception in the muscle (Smith et al., 1994).

POSSIBLE RISKS OF PARTICIPATION AND EFFORTS TO MINIMIZE RISK

Possible risks to the subjects during the cycle test include fast or irregular heart beats, dizziness, fainting, and remote possibility of heart attack, stroke, or sudden death. Orthopedic injuries such as strains and sprains are also possible. Risk will be minimized by having the tests monitored by personnel trained in administering graded exercise tests. Ms. Janet Wojcik is certified by the American College of Sports Medicine as a Health/Fitness

Instructor and has completed an internship in the Cardiac Therapy and Intervention Center at Virginia Tech.

Possible risks to the subjects during the resistance exercise on the Biodex System include orthopedic trauma such as strains and sprains. This will be minimized by having trained personnel conduct the evaluation. In addition, acute muscle injury is rare during use of machines such as the Biodex when compared to Universal weight training stations and free weights. The Biodex will automatically relieve the stress on the muscle if the muscle cannot generate enough force. Fatigue will occur during the test. Delayed onset muscle soreness (DOMS) is expected to occur in the exercised leg within 24 hours following the intense exercise bout on d 3 and will persist for several days.

Possible risks to the subjects during the muscle biopsy include fainting, infection, or allergic reaction to the anesthetic. All subjects will be questioned verbally and in writing about any reactions to anesthetics they may have had in the past (e.g., at the dentist). Risk of fainting will be minimized by having the subjects recline during and for 20 minutes after the procedure. Any subject complaining of symptoms such as dizziness or nausea will be asked to remain in the lab and will be observed until the symptoms subside. Risk of infection will be minimized by maintaining a sterile environment during the biopsy procedure.

We recently completed a trial of the muscle biopsy procedure with no complications. Ms. Rinehart, the certified laboratory technician performing the biopsy, has been trained by Dr. Joseph Houmard of East Carolina State University, a professor with experience in over 500 muscle biopsies. She was supervised by him during our trials of the biopsy. In addition, she will be supervised by a physician during all biopsy procedures.

Possible risks to the subjects during blood draws include infection and bruising. This will be minimized by having Ms. Rinehart perform all blood draws. Subjects will remain in the laboratory for 20 min following the blood draw so they can be monitored.

To minimize risks to the experimenters from blood borne pathogens, universal precautions for potentially infectious biological materials will be employed. This will include use of sterile gloves by Ms. Rinehart as well as any experimenters who will handle blood or tissue samples. All biological wastes will be disposed of in an appropriate container.

A phone is available in the testing laboratory in case of need for emergency medical assistance.

BENEFITS OF PARTICIPATION

Results of the subjects' strength test and aerobic fitness cycling test will be shared with them upon their request. Assuming external funding for the project, all subjects will be paid $150 for their participation in the study.

FREEDOM TO WITHDRAW

All subjects have freedom to withdraw from the experiment at any time without prejudice.

CONFIDENTIALITY

Subjects will not be identified in any discussion of the procedures or results.

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Appendix C

Informed Consent

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants
of Investigative Projects

TITLE:

Effect of Beverage on Glycogen Resynthesis and Extent of Muscle Damage Induced by Resistance Exercise

PURPOSE AND BACKGROUND

Much of the research concerning nutrition and athletic performance has focused on foods eaten prior to and during exercise. However, nutrition following exercise may be important for athletes who participate in multiple workouts or competitions in one day or over consecutive days. This study will evaluate consumption of three different beverages on recovery from an intense bout of weight training exercise.

PROCEDURES

Prior to the start of the experimental period, you will undergo three performance tests. A practice session for both performance tests will allow you to get familiar with the laboratory setting and procedures. At this practice session your height and weight will be taken, and your body fat percentage will be measured with skinfold calipers. Your skin will be grasped by calipers at the chest, abdomen, and thigh, and these measurements may pinch or tickle somewhat.

The first performance test consists of pedaling a stationary bicycle beginning from a light intensity with the resistance on the cycle increasing until you can no longer keep the pedaling pace. For 4 hours prior to the test you should consume no food or drink beverages containing caffeine (cola products, teas, coffee, Mountain Dew). During the test, a mouthpiece will be placed in your mouth to collect expired oxygen and carbon dioxide in order to calculate maximum oxygen uptake, or VO_{2max}. Your nose will be pinched with a noseclip similar to one used by swimmers. Your mouth will feel dry following the test since your nose, which has been closed, adds moisture to the air you breathe. Since your heart rate will be monitored by electrocardiogram, 3 electrodes will be placed on your torso. These electrodes detect heart muscle activity. They do not emit any electricity, and you will not feel any pain from these electrodes. Small patches (quarter-size) of chest hair may need to be shaved in order to properly place the electrodes on your skin. Surface oil on the skin may need to be removed by light scrubbing with a towel. This may make the skin temporarily pink.

The second performance test consists of finding how much muscular force can be produced from your thigh muscles. This test will be performed on a Biodex machine, which looks similar to a Nautilus or Universal leg extension weight lifting station. This type of machine is widely used in sports settings because it can be used to test for muscle strength, train for muscular fitness improvements, and to rehabilitate injuries. You will sit down in a

chair attached to the Biodex machine, and straps will hold you in place on the chair: over your hips on the seat of the chair and into a pad which covers your shin. This shin pad is attached to an arm which connects to the measurement device in the machine. You will be asked to perform 5 maximal leg extension "kicks" with this leg. Your leg will be assisted back to the starting position between repetitions. This type of testing is very safe since the machine's resistance will adjust to what you perform. The peak muscular force will be determined following this brief test. You will repeat this test on days 6, 7, and 8 of the study.

The final performance test will be performed on a Nautilus leg extension weightlifting machine and will assess the one-repetition maximum strength of the same thigh. This is the amount of weight you can lift with only 1 repetition. You will sit down in the chair attached to the weight stack, and a strap will hold your hips on the chair. You will be asked again to perform leg extension kicks with your leg. The test will terminate when you can only perform 1 repetition at a given weight through the full range of motion. We ask that you perform no other exercise, aerobic or strength, outside what is part of the study.

The first 2 days of the 10-day study will allow you to get used to the controlled diet. Throughout the study, you will report for breakfast and dinner at the Nutrition Department's feeding area, located on the third floor of Wallace Hall. In addition, you will be given a cooler which contains your lunch and snacks to take with you. It is important that you eat no other foods or take no vitamin supplements or medications without checking with study personnel during the course of the project. It is also important that you drink no alcohol or use recreational drugs during the course of the study. We are able to screen your blood and urine samples for alcohol/drugs.

Starting on Day 3 you will need to collect all your urine. A plastic jug with preservative will be given to you daily and returned to the lab the following morning. Each collection day begins with the second urination of the day. Morning blood draws will begin on the morning of Day 4 and will continue until the morning of Day 10. Blood samples will be taken from your arm by Ms. Janet Rinehart, a certified medical laboratory technician in the Department of Human Nutrition and Foods.

On the evening of Day 4 you will report to the human performance laboratory to cycle for 30-45 minutes at an intensity of 70% of your maximum oxygen uptake, a moderately-high intensity, determined from the stationary cycling test. Following this training bout you will perform 5 sets of 1 minute maximal sprints on the cycle interspersed with 2 minutes of light recovery. You will be allowed to have as much water as you wish during the ride, and you will be allowed to take rest breaks. Fatigue is expected to occur during the ride. Following the ride you will be given a low-carbohydrate snack. This snack will delay replacement of muscle carbohydrate stores, and you may feel somewhat sluggish during the rest of the evening into the following morning.

On Day 5 of the experimental period, you will report to the small weight room in Cassell Coliseum after an overnight fast. Blood samples will be taken from your arm by Ms. Rinehart. Following this first blood draw you will perform 10 sets of 10 repetitions of eccentric leg curls on the Nautilus machine, where you must resist the machine with your thigh muscles as it tries to pull your leg downward. You will be asked to lower the leg for a 4 second count. Your leg will be assisted back to the starting position between repetitions. Fatigue is expected to occur during this exercise test. This test allows us to obtain muscle responses that would ordinarily be seen after an intense weight training bout. It is expected you will experience muscle soreness in the exercised leg beginning within 24 hours of this exercise bout and lasting for up to several days. It is important not to take any over-the

counter medications such as aspirin, Tylenol, ibuprofen (Advil, Motrin), naproxen sodium (Alleve), or ketoprofen (Actron, Orudis KT) during the course of this muscle soreness.

Immediately following the exercise session on Day 5, you will undergo another blood draw as well as a muscle biopsy procedure performed by Ms. Rinehart. She will be observed by a physician. You will be asked to lie on a flat examination table in the training room. The hair on your dominant leg will be shaved and the leg cleaned with iodine (will make the skin temporarily yellow). A sterile drape will be placed over the skin site. A substance used to cool the skin (ethyl chloride) will be sprayed on the skin in order to numb the area. A local anesthetic, lidocaine, will be injected over the site in several punctures. This procedure may sting, similar to a bee sting. After the area is numb, a small incision about 1/2 inch wide will be made in the skin with a scalpel blade. Pressure will be applied with a sterile gauze pad to reduce bleeding. Then the sterile biopsy needle about the diameter of a pencil (1/4 inch) will be inserted about 1 inch into your thigh. A piece of muscle about half the size of a pencil eraser (25-100 mg) will be removed. The time required to insert the needle and remove the sample is about 3-5 seconds. Following removal of the needle, pressure will be applied to the area for 20 minutes to reduce bleeding and swelling. The incision will be closed with a sterile strip and a Band-Aid; it does not require stitches. You will have a small scar, which will fade with time, as a result of the incision.

This muscle biopsy procedure will be repeated on the same leg at 24 (Day 6) and 72 hours (Day 8) after the first sample. Following these second and third samples, you will be asked to remain in the laboratory for at least 20 minutes so we can see that you feel okay. You will be instructed to keep the pressure wrap on the leg for 8 hours after the biopsy, and the steri-strip and Band-Aid on for 3 days. When you leave you will be encouraged to use the leg, not "baby" it. This will reduce stiffness. You will be asked to keep the site clean, as with any cut, to reduce risk of infection. Following the last biopsy, you will be asked to return to the lab 3 days later so we can examine the site for appropriate healing.

Immediately following the muscle biopsy, you will be given a beverage to drink. Two hours later you will be given the same beverage. Additional blood samples will be taken at 3 and 6 hours after the intense exercise bout on Day 5 as well as days 6-10 following an overnight fast. The morning blood samples will be taken just prior to breakfast at Wallace Hall. You will be asked to remain the laboratory for 20 min after each blood draw so we can check that you are okay.

At the time of the daily blood draw and urine collection, you will be shown a 1-10 scale and asked to rate your muscle soreness in the exercised leg (1 = no soreness, 10 = unbearable soreness).

POSSIBLE RISKS OF PARTICIPATION AND EFFORTS TO MINIMIZE RISK

Possible risks during the cycle test include fast or irregular heart beats, dizziness, fainting, and remote possibility of heart attack, stroke, or sudden death. Orthopedic injuries such as strains and sprains are also possible. Risk will be minimized by having the tests monitored by personnel trained in administering graded exercise tests.

Possible risks during the resistance exercise on the Biodex System include orthopedic trauma such as strains and sprains. Again, this is unlikely because the machine's resistance will adjust to what you perform. In addition, trained personnel will conduct the evaluation.

Possible risks during the resistance exercise on the Nautilus machine include orthopedic trauma such as strains and sprains. Fatigue will occur during the test. Delayed onset muscle soreness (also known as DOMS) is expected to occur in the exercised leg within 24 hours following the intense exercise bout on Day 5, and this soreness will persist for several days.

Possible risks during the muscle biopsy include fainting, infection, or allergic reaction to the anesthetic. Risk of fainting will be minimized by having you recline during and for 20 minutes after the procedure. Risk of infection will be minimized by maintaining a sterile environment during the biopsy procedure. You will be given instructions on keeping the site clean to minimize infection. We recently completed a trial of the muscle biopsy procedure on several individuals with no adverse reactions. Ms. Rinehart, the certified laboratory technician performing the biopsy, has been trained by a professor with experience in over 500 muscle biopsies. In addition, she will be supervised by a physician during the procedure.

Possible risks during blood draws include infection and bruising. This will be minimized by having Ms. Rinehart perform all blood draws. You will be asked to remain in the laboratory for 20 min following the blood draw so we can see that you are okay.

Universal precautions will be taken in the handling of all blood, urine, and body fluids.

A phone is available in all testing areas in case of need for emergency medical assistance.

BENEFITS OF PARTICIPATION

If you wish, we will share data regarding your muscular strength obtained from the Biodex testing, your aerobic fitness obtained from your stationary cycling test, and your body fat percentage. Upon completion of the study, you will receive a bonus of $150.

FREEDOM TO WITHDRAW

You may withdraw your consent to participate at any time.

CONFIDENTIALITY

Your name will not be used in any future discussion of the procedures or in the results of the analysis.

SUBJECT'S RESPONSIBILITIES

If you are selected for the study, it is your responsibility to:

1. Inform the experimenters of any allergy to "cain" type anesthetics, presence of HIV or Hepatitis B infection, or presence of any other communicable disease that could be transmitted to others.
2. Comply with dietary and exercise guidelines during the course of the experimental period.

3. Consume no vitamin or mineral supplements as well as consume no over-the-counter pain relief tablets.

4. Consume no alcoholic beverages and/or refrain from use of recreational drugs during the experimental period.

5. Inform the experimenters of any unusual symptoms such as dizziness or nausea.

6. Remain in the laboratory for 20 minutes following the muscle biopsy procedure and blood withdrawal, leave the pressure wrap from the biopsy on for 8 hours, and leave the steri-strip and Band-Aid on for 3 days after the biopsy procedure.

7. Inform the experimenters of any unusual soreness or inflammation in the biopsy area in the days following the procedure.

8. Submit to an HIV and/or Hepatitis B test if study personnel become exposed to your blood.

APPROVAL OF RESEARCH PROCEDURE

This procedure has been approved, as required, by the Institutional Review Board (IRB) for projects involving human subjects at Virginia Tech.

SUBJECT'S PERMISSION

I, (print name) _____ have read and understand the informed consent and conditions of this procedure. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent for participation in this study.

Signature of Participant:

_____ Date:_____

Should I have any questions about this procedure or its conduct, I can contact:

Janet Wojcik, M.S.
Doctoral Candidate
Department of Human Nutrition and Foods
231-4900 (office), 552-2953 (residence)

Janet Walberg Rankin, Ph.D.
Associate Professor
Department of Human Nutrition and Foods
231-6355

Ernest Stout, Ph.D.
Chairman of the IRB at Virginia Tech
231-9359

Appendix D

Initial Screening Form

Nutrition and Exercise Initial Screening Form

Date:

Please state your age:

Please state your height and weight:

Where do you live? campus / Blacksburg / Christiansburg / Mont. Co. / Other_____

Have you been a subject in a research study before? **Y / N**
If yes, please describe.

Will you be out of town for any period from April 1 through May 10? **Y / N**
If yes, please describe dates.

Will you be in town for the first summer session? **Y / N**
If yes, please describe.

Do you exercise now? **Y / N**
If yes, please describe your current exercise program (what activities and how often):

What other physical activities have you participated in? When was the last time you participated?

Has a doctor ever said you had a heart problem? **Y / N**
If yes, please describe.

Do you have or have you had high blood pressure? **Y / N**
If yes, please describe.

Do you have diabetes or any other chronic illness? **Y / N**
If yes, please describe.

Have you had any recent illnesses or hospitalizations other than a cold? **Y / N**
If yes, please describe.

Do you have any breathing or cardiovascular problems which would prevent you from performing aerobic exercise on a stationary cycle? **Y / N**
If yes, please describe.

Do you have any orthopedic or joint problems which would prevent you from exercise?
Y / N If yes, please describe.

Do you have lactose intolerance (milk allergy) or other food/medication allergies?
Y / N
If yes, please describe.

Have you ever fainted when you have given blood? **Y / N**

Do you feel faint or faint at the sight of blood? **Y / N**

Are you allergic to local anesthetics such as those you receive at the dentist? **Y / N**

Name:_____ **Daytime Phone:_____**
Evening Phone:_____
e-mail:_____

Appendix E

Medical and Health History Form

VIRGINIA TECH LABORATORY FOR HEALTH AND EXERCISE SCIENCE
DEPARTMENT OF HUMAN NUTRITION, FOODS, AND EXERCISE
MEDICAL AND HEALTH HISTORY

Name: _____ Age: _____ Birth Date: _____

Student ID:_____

Local Address: _____

City, State, ZIP:_____

Local Phone Number: _____(home)_____(office/other--specify)

Permanent Address:_____

City, State, ZIP:_____

Person to contact in case of emergency: _____

Relationship: _____ Phone: _____

Primary Care Physician: _____ Phone: _____

Medical History

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician that you had:

	Yes	**No**
Heart disease or any heart problem	_____	_____
Rheumatic fever	_____	_____
Respiratory disease or breathing problems	_____	_____
Circulation problems	_____	_____
Kidney disease or problems	_____	_____
Urinary problems	_____	_____
Reproductive problems	_____	_____

Musculoskeletal problems	_____	_____
Fainting or Dizziness	_____	_____
High Cholesterol	_____	_____
Diabetes	_____	_____
Thyroid problems	_____	_____
High blood pressure	_____	_____
Lactose Intolerance (milk allergy)	_____	_____
Allergies to Local Anesthetics	_____	_____
Other Allergies	_____	_____

If "yes" to any of the above, please indicate the date, explain, and describe:

_____
_____
_____
_____
_____
_____
_____
_____

Please list any hospitalizations/operations/recent illnesses (date/type):

_____
_____
_____
_____
_____
_____

Please list all medications (prescription and over-the-counter) you are currently taking or have taken within the past week:

_____
_____
_____
_____
_____
_____

Please list any vitamins or other nutritional supplements you are currently taking or take on a regular basis:

_____
_____
_____
_____
_____
_____

Exercise Habits

Please describe the activities you currently participate in or have participated in the past.

	Curr. or Past (✓)		How Often? (minutes/wk)	Date Last Participated
Running	_____	_____	_____	_____
Weight Training	_____	_____	_____	_____
Road Cycling	_____	_____	_____	_____
Mountain Biking	_____	_____	_____	_____
Rowing	_____	_____	_____	_____
Swimming	_____	_____	_____	_____
Racquet Sports	_____	_____	_____	_____
Hiking	_____	_____	_____	_____
Volleyball	_____	_____	_____	_____
Basketball	_____	_____	_____	_____
Team Sport:_____	_____	_____	_____	_____
Other:_____		_____	_____	_____
Other:_____		_____	_____	_____

Do you ever feel faint, short of breath, or chest discomfort with exercise?
Yes_____ No_____ If "yes", please describe:_____

Are there any orthopedic limitations you have which may restrict your ability to perform exercise of moderate to high intensity? Yes_____ No____
If "yes", please describe:
_____

Family History

Has anyone in your family been diagnosed or treated for any of the following:

	Yes	No	Relationship	Age (at occurrence)
Heart attack	____	____	_____	_____
Heart disease	____	____	_____	_____
High blood pressure	____	____	_____	_____
Stroke	____	____	_____	_____
Kidney disease	____	____	_____	_____
Diabetes	____	____	_____	_____

Dietary Habits

Please list your current height: _____(feet and inches)
Please list your current weight: _____(lbs.)
What would you like to weigh? _____(lbs.)
What is the most you have weighed since age 18: _____(lbs.)
What is the least you have weighed since age 18: _____(lbs.)
Has your weight fluctuated more than 5 lbs. in the past year? **Y or N**

If yes, how much? _____(lbs)
If yes, was this weight loss on purpose? **Y or N**

Which do you eat regularly (indicate with **Y** for Yes, **N** for No, **O** for Occasionally)
Breakfast_____ Midmorning snack_____
Lunch_____ Midafternoon snack_____
Dinner_____ Evening snack_____
How long does it take for you to eat a meal?_____minutes

How often do you go out to eat per week?_____times
How often do you eat red meat per week?_____times
How often do you eat chicken per week?_____times
How often do you eat fish per week?_____times
How often do you eat dessert per day?_____times_____times per week
How often do you eat fried foods per week?_____times

How much coffee/tea do you drink per day?_____cups
What do you add to your coffee/tea and how much?_____
How many soft drinks (cans) do you consume a day?_____cans
What type and brands of soft drinks do you consume (indicate diet/non-diet):_____
_____
_____

Do you consider yourself a vegetarian? **Y or N**
If yes, do you consume any animal products? **Y or N**

Do you eat while doing other activities such as studying or watching TV? **Y or N**
When you snack, how many *times per week* do you eat the following?

cookies_____	candy_____
cakes_____	pies_____
doughnuts_____	pretzels/potato chips_____
chips & salsa/nachos_____	cheese/crackers_____
ice cream/frozen yogurt_____	peanuts/nuts_____
pizza_____	

other
(describe):_____
_____

Who does most of the cooking in your household? (Please circle) You spouse/signif. other
parent roommates
n/a: on meal plan

Do you have any specific food allergies? **Y or N**
If yes, please describe:_____
_
_____
_____

Smoking Habits

Do you currently smoke cigarettes or cigars? **Y or N**
If yes, how much per day? _____cigarettes or cigars
Have you quit smoking? **Y or N**

If yes, when did you quit? _____(give date)
Do you use smokeless tobacco? **Y or N**
If yes, how much do you use per day? _____(give amount)

Drinking Habits

During the past month, how many days did you drink alcoholic beverages? _____days
During the past month, how many times did you have 5 or more drinks? _____times
On average, how many glasses of the following beverages do you consume in a week?

Beer	_____ glasses or cans
Wine	_____glasses
Mixed Drinks	_____glasses
Shots	_____number
Other (specify):	_____number

Miscellaneous

	Yes	**No**
Do you feel faint when you have your blood drawn?	_____	_____
Do you feel faint at the sight of other people's blood?	_____	_____
Will you be out of town any time before May 10?	_____	_____
Will you be here for first summer session (5/20-7/1)?	_____	_____

If "yes" to any of above, please describe:_____
_____
_____
_____
_____
_____

Schedule
Please list your class schedule (class, room, and times)

Mon	Tues	Wed	Thurs	Fri

Please list your work schedule:

Mon	Tues	Wed	Thurs	Fri	Sat	Sun

Please describe which part of the study you feel will be the most difficult for you to comply with.

Please sign below to indicate the above information is correct.

_____	_____
Print Name	**Signature** **Date**

All information is confidential

Appendix F

Time Schedule Given to Subjects

Schedule for All Meals and Tests
Nutrition and Exercise Study

Here is your schedule of what tests you will undergo, where you will need to be, and what time you will need to be there. If any questions, don't hesitate to call Janet Wojcik at 231-4900 or 552-2953.

Study Days 1 and 2. Be prepared to receive a phone call 30 minutes prior to your first appointment every morning.

❒	Body weight--all	8:15 a.m.	Wallace Hall, Room 332-334
❒	Breakfast--all	8:30	Wallace Room 334
❒	Bag Lunch	Noon	On your own
❒	Dinner	5:00 p.m.	Wallace Room 334
❒	Bag Snacks	as needed	On your own

Study Day 3

❒	Body weight--all	8:00 a.m.	Wallace Hall
❒	Pick up urine collection bottles, begin collecting with second urination	8:05	Wallace Hall
❒	Breakfast--all	8:15	Wallace, 334
❒	Bag Lunch	Noon	On your own
❒	Dinner	5:00 p.m.	Wallace, 334
❒	Bag Snacks	as needed	On your own

Study Day 4

❒	Body weight	8:00	Wallace Hall
❒	Turn in/pick up urine collection bottles keep collecting	8:00	Wallace Hall
❒	First blood draw--Subject #1	8:05 a.m.	Wallace Hall Blood Draw Room
❒	First blood draw--Subject #2	8:10 a.m	same as above
❒	First blood draw--Subject #3	8:15 a.m.	
❒	First blood draw--Subject #4	8:20 a.m.	

Study Day 4 continued

❒ Breakfast--all	8:30 a.m.	Wallace
❒ Bag Lunch	Noon	On your own
❒ Bag Dinner	4-5:00 p.m.	
❒ Bag Snacks	as needed	On your own
❒ Endurance Cycling--Subjects #1 & #2	7:30 p.m.	WMH 230
❒ Endurance Cycling-- Subjects #3 & #4	8:45 p.m.	WMH 230
❒ Eggs and peanuts snack	9:00 p.m.	WMH or home
❒ Eggs and peanuts snack	10:00 p.m.	

Study Day 5

❒ Weight/Urine Coll.-- Subject #4	7:30 a.m	Cassell Coliseum Training Room
❒ Weight/Urine Coll.--Subject #1	7:50 a.m.	
❒ Weight/Urine Coll.-Subject #2	8:20 a.m	Cassell training room
❒ Weight/Urine Coll.--Subject #3	8:50 a.m.	
❒ Blood/soreness-- Subject #4	7:30 a.m.	Cassell Training Room
❒ Blood/soreness--Subject #1	8:00 a.m.	Cassell Training Room
❒ Blood/soreness--Subject #2	8:20 a.m.	
❒ Blood/soreness--Subject #3	8:50 a.m.	
❒ Resistance Exer-- Subject #4	7:40 a.m.	Cassell Coliseum Weight Room
❒ Resistance Exer--Subject #1	8:10 a.m	
❒ Resistance Exer--Subject #2	8:40 a.m.	
❒ Resistance Exercise--Subject #3	9:00 am.	
❒ Sore/Blood/1st Muscle-- Subject #4	8:00 a.m.	Training Room
❒ Sore/Blood/1st Muscle--Subject #1	8:30 a.m.	Training Room
❒ Sore/Blood/1st Muscle--Subject #2	9:00 a.m.	Training Room
❒ Sore/Blood/1st Muscle--Subject #3	9:25 a.m.	Training Room
❒ Consume Beverage-- Subject #4	8:30 a.m.	Training Room
❒ Consume Beverage--Subject #1	9:00	Training Room
❒ Consume Beverage--Subject #2	9:30	Training Room
❒ Consume Beverage--Subject #3	10:00	Training Room
❒ Drink Beverage #2-- Subject #4	10:30 a.m.	On your own
❒ Drink Beverage #2--Subject #1	11:00 a.m.	
❒ Drink Beverage #2--Subject #2	11:30	
❒ Drink Beverage #2--Subject #3	Noon	
❒ Blood Draw-- Subject #4	11:30 a.m.	
❒ Blood Draw--Subject #1	11:50 a.m.	Wallace Hall
❒ Blood Draw--Subject #2	12:45	
❒ Blood draw--Subject #3	1:00 p.m.	

❒	Lunch-- Subject #4	11:30	
❒	Lunch--Subject #1	Noon	Wallace 334
❒	Lunch--Subject #2	1:00 p.m.	
❒	Lunch--Subject #3	1:10 p.m.	
❒	Blood Draw-- Subject #4	2:30 p.m.	
❒	Blood Draw--Subject #1	3:00 p.m.	Wallace, third floor
❒	Blood Draw--Subject #2	3:45 p.m.	Wallace, third floor
❒	Blood draw-Subject #3	4:00 p.m.	
❒	Dinner	5:00 p.m.	to go--pick up at blood draw
❒	Bag Snacks		as needed, anytime after Lunch

Study Day 6

❒	Weight/Urine collection-- Subject #4	7:20 a.m.	WMH 228
❒	Weight/Urine collection--Subject #1	7:50 a.m.	WMH 228
❒	Weight/Urine collection--Subject #2	8:20 a.m.	
❒	Weight/Urine collection--Subject #3	8:50	
❒	Biodex-- Subject #4	7:30 a.m	WMH 228
❒	Biodex--Subject #1	8:00 a.m.	WMH 228
❒	Biodex--Subject #2	8:15 a.m.	
❒	Biodex--Subject #3	9:00	
❒	blood/muscle--Subject #4	8:00 a.m.	Cassell Coliseum Training Room
❒	blood/muscle--Subject #1	8:30 a.m.	Training Room
❒	blood/muscle--Subject #2	9:00 a.m.	
❒	blood/muscle--Subject #3	9:30	
❒	Breakfast--Subject #4	8:40 a.m.	Wallace 334
❒	Breakfast-Subject #1	9:00	Wallace 334
❒	Breakfast--Subject #2	9:30	Wallace 334
❒	Breakfast--Subject #3	10:00 a.m.	Wallace 334
❒	Bag Lunch	Noon	On your own
❒	Dinner	5:00 p.m.	Wallace
❒	Bag Snacks	as needed	On your own

Study Day 7

❒	Weight/Urine collection--Subject #1	7:20 a.m.	WMH 228
❒	Weight/Urine collection--Subject #4	7:40 a.m.	WMH 228
❒	Weight/urine collection--Subject #2	8:00 a.m.	
❒	Weight/urine collection--Subject #3	8:20 a.m.	
❒	Biodex--Subject #1	7:30 a.m	WMH 228
❒	Biodex--Subject #4	7:50 a.m.	WMH 228
❒	Biodex--Subject #2	8:10 a.m.	
❒	Biodex--Subject #3	8:30 a.m.	

❒	Blood--Subject #1	8:00 a.m.	Wallace Blood Draw Room
❒	Blood--Subject #2	8:30 a.m.	
❒	Blood-Subject #3	8:40 a.m.	
❒	Breakfast--Subject #1	8:15	
❒	Breakfast--Subject #4	8:20	
❒	Breakfast--Subject #2	8:40	Wallace, 334
❒	Breakfast--Subject #3	8:45	
❒	Bag Lunch	Noon	On your own
❒	Dinner	5:00 p.m.	Wallace
❒	Bag Snacks	as needed	On your own

Study Day 8

❒	Weight/Urine collection--Subject #4	7:20 a.m.	WMH 228
❒	Weight/Urine collection--Subject #1	7:50 a.m.	WMH 228
❒	Weight/Urine collection--Subject #2	8:20 a.m.	
❒	Weight/Urine collection--Subject #3	8:50 a.m.	
❒	Biodex--Subject #4	7:30 a.m	WMH 228
❒	Biodex--Subject #1	8:00 a.m.	WMH 228
❒	Biodex--Subject #2	8:15 a.m.	
❒	Biodex--Subject #3	9:00 a.m.	
❒	blood/muscle--Subject #4	8:00 a.m.	Cassell Coliseum Training Room
❒	blood/muscle--Subject #1	8:30 a.m.	Training Room
❒	Blood/muscle--Subject #2	9:00 a.m.	
❒	blood/muscle--Subject #3	9:30 a.m.	
❒	Breakfast-- Subject #4	8:40 a.m.	Wallace 334
❒	Breakfast-Subject #1	9:00	Wallace 334
❒	Breakfast--Subject #2	9:30	Wallace 334
❒	Breakfast--Subject #3	10:00 a.m.	Wallace 334
❒	Bag Lunch	Noon	On your own
❒	Dinner	5:00 p.m.	Wallace
❒	Bag Snacks	as needed	On your own

Study Day 9 (next wave of participants also begins)

❒	weight/soreness	8:00	Wallace Hall, 332
❒	Urine collection--all	8:00	Wallace Hall
❒	blood draw--Subject #1	8:05 a.m.	Wallace Blood Draw Room
❒	blood draw--Subject #2	8:10 a.m	same as above
❒	blood draw--Subject #3	8:15 am.	
❒	blood draw--Subject #4	8:20 a.m.	

❒	Breakfast--all	8:30	Wallace, third floor
❒	Bag Lunch	Noon	On your own
❒	Dinner	5:00 p.m.	Wallace
❒	Bag Snacks	as needed	On your own

Study Day 10

❒	Body weight/soreness	8:00 a.m.	Wallace Hall, 332
❒	Turn in final urine Samples	8:00 a.m.	Wallace
❒	FINAL blood draw--Subject #1	8:05 a.m.	Wallace Blood Draw Room
❒	FINAL blood draw--Subject #2	8:10 a.m	same as above
❒	FINAL blood draw--Subject #3	8:15 am.	
❒	FINAL blood draw-- Subject #4	8:20 a.m.	

Appendix G

Body Weight and Anthropometric Data Recording Form

Body Weight and Anthropometric Data

Subject:_____ Age:_____ Height:_____in. ___cm_____m

Weight:_____kg _____lb.

BMI

Weight (kg)/Height (m^2) = _____/_____ = _____

Skinfold Data

date:_____

Average

Chest _____ _____ _____ _____

Abdomen _____ _____ _____ _____

Thigh _____ _____ _____ _____

Sum of Skinfolds: _____

% Body Fat: _____

Body Weight (no shoes, indoor clothing)

Day	Weight (Kg)	Time
Pre-test		
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Appendix H

Exercise Testing Forms

Biodex Strength Testing

Subject:_____ Dominant Leg: **R** or **L**

Seat Forward Position:_____ Seat Back Position:_____

Lever Arm Length: _____ Dynamometer Position:_____

Joint Ranges:
Extension:____
Flexion: ____

***Have subject perform 5 min warm-up on Monarch cycle at 0.5 kg**

Test	Date	Time	Peak Quadriceps Concentric Torque (ftlbs)	Work per Max Repetition (ftlbs)	Total Work per Set of 5 repetitions (ftlbs)
Pre-test					
D6 (24 h post-eccentric exercise)					
D7 (48 h post-eccentric exercise)					
D8 (72 h post-eccentric exercise)					

Nautilus One-Repetition Maximum (1-RM) Protocol

Subject:_____ Age:_____ Weight:_____

Date:_____ Time:_____ Dominant Leg: **R** or **L**

Weight	**# Repetitions**

1-RM: _____

120% 1-RM: _____
(resistance for eccentric exercise)

Isotonic Eccentric Exercise Protocol

Subject:_____ Age:_____ Weight:_____

Date:_____ Time:_____

Set	# Repetitions	Time Begin	Time End
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Instructions For Aerobic Capacity Test

Here are some instructions so you can be best prepared for the maximal aerobic capacity test. During this test you will be asked to pedal a stationary cycle beginning from a light resistance with the resistance increasing until you can no longer keep the pedaling pace. Electrodes will be placed on your torso to monitor your heart rate, and a mouthpiece will be placed in your mouth to collect expired air. Total time in the laboratory will be approximately 30-45 minutes.

Before the aerobic capacity test:

- Report to the laboratory in Room 230 War Memorial Hall at your designated time: _____a.m. or p.m. Date:_____
- Refrain from eating, drinking, smoking (or using chewing tobacco), or using caffeinated beverages (coffee, teas, colas, Mountain Dew) for 4 hours prior to testing
- Avoid exercise for 24 hours prior to the test
- Do try to drink at least 4-6 glasses (8 oz.) of water the day before the test and the day of the test
- Bring shorts, T-shirt, sneakers, towel. We recommend padded cycling shorts if you have them
- Try to gain a good night's rest prior to the testing day
- Bring your toiletries if you wish to shower after the test
- Call us to reschedule if you are not feeling well (e.g. if you have a cold, sore throat, fever, body aches, upset stomach)

If you have any questions regarding the testing contact:
Janet Wojcik, M.S.
120 War Memorial Hall
231-4900 office
552-2953 residence
email: jwojcik@vt.edu

VO_{2peak} Protocol

Subject:_____ Date:_____ Time:_____

Age:_____ Weight:_____ Height:_____ Technician (s):_____

Cycle Number: _____ Cycle Seat Height: _____

Resting Data:

Time	HR	RPE (Overall/Legs)
1:00		
2:00		

Exercise Data:

Time	Resistance on Cycle	HR	RPE (O/L)
1:00	0.5 kg		
2:00	0.5 kg		/
3:00	1.0kg		
4:00	1.0 kg		/
5:00	1.5 kg		
6:00	1.5 kg		/
7:00	2.0 kg		
8:00	2.0 kg		/
9:00	2.5 kg		
10:00	2.5 kg		/
11:00	3.0 kg		
12:00	3.0 kg		/
13:00	3.5 kg		
14:00	3.5 kg		/
15:00	4.0 kg		
16:00	4.0 kg		/
17:00	4.5 kg		
18:00	4.5 kg		/
19:00	5.0 kg		
20:00	5.0 kg		/
PEAK time:			/

Recovery:

1:00	
2:00	
3:00	
4:00	

Instructions For Endurance Ride

Here are some instructions so you can be best prepared for the endurance bike ride. During this test you will pedal a stationary cycle at a moderately-high intensity for 30-45 minutes. The intensity will get higher the longer you ride. You will then perform 5 sets of 1 minute sprints as hard as you can go with 2 minutes of light exercise in between each sprint. Total time in the laboratory will be approximately 1 hour. During the ride, a heart rate monitor will be placed around your torso.

Before the evening endurance ride:

- Report to the laboratory in 230 War Memorial Hall at your designated date:_____and time:_____p.m.
- Bring shorts, T-shirt or tank top, sneakers, towel. We recommend cycling shorts if you have them
- It is OK to bring a Walkman, CD, or cassette tape (we have boomboxes)
- Bring a water bottle if you have a favorite one you like to use
- Bring your toiletries if you wish to shower afterwards
- **Do not eat any snacks between dinner and the time of the endurance ride**
- **You must have no other snacks to eat after the ride besides the peanuts and eggs**
- Do not drink any caffeine (coffee, teas, colas, Mountain Dew) or use tobacco products for 4 hours prior to the ride

If you have any questions regarding the testing contact:
Janet Wojcik, M.S.
120 War Memorial Hall
231-4900 office
552-2953 residence
email: jwojcik@vt.edu

Aerobic Glycogen Reducing Protocol

Subject:_____ Date:_____ Time:_____

Age:_____ Weight:_____ Height:_____ Technician(s):_____

Cycle Number: _____ Cycle Seat Height: _____

Peak VO_2:_____ml/kg/min

70% VO_{2peak} for glycogen reduction:_____ml/kg/min

Corresponding Resistance on Cycle Ergometer:_____kg

Corresponding Heart Rate:_____beats/min

Exercise Data:

Time	HR (beats/min)	RPE (Overall/Legs)
5:00		/
10:00		/
15:00		/
20:00		/
25:00		/
30:00		/
35:00		/
40:00		/
45:00		/

Sprints (1 min all-out at Kg above, 2 min active recovery 1.0 Kg):

Sprint	Time Begin	Time End
1		
2		
3		
4		
5		

Rest Periods:

Time Beginning	Time End

Borg Rating of Perceived Exertion Scale

6

7 Very, Very Light

8

9 Very Light

10

11 Fairly Light

12

13 Somewhat Hard

14

15 Hard

16

17 Very Hard

18

19 Very, Very Hard

20

Appendix I

Diet Record Forms

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 1

BREAKFAST

5534	Cereal-Corn Flakes-Kelloggs-CNF	2.00 CUPS x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
278	Orange Juice	1.00 CUP x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
121	Three-Bean Salad	16 oz. x_____
105	Butter	10 g (2 pats) x_____

DINNER

1826	Salad-Green Salad-Tossed	200 g x_____
5445	Salad Dressing-Italian-Regular	2.00 TBSP x_____
332	Bread-Italian	1.00 SLICE x_____
10005	Pasta & Sauce/Italian Cheese/Lipton	2.00 SERVINGS x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
573	Green Beans	1.00 CUP x_____

SNACKS/OTHER

4911	Bagel-plain-toasted	1 ITEM x_____
4979	Jelly	1.00 TBSP x_____
105	Butter	5.00 g x_____
417	Cookie-Fig Bar	4.00 ITEMS x_____
223	Apples-Raw-With Skin-2 ¾ inch diameter	1.00 ITEM x_____
523	Peanuts	0.25 CUP (36 g) x_____
5221	Egg-Hard Boiled	140 g x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 2

BREAKFAST

5230	Egg-Chicken-Whole Scramble-Milk/Margarine	1.50 CUPS x_____
105	Butter	10 g (2 pats) x_____
551	Jelly-Regular	2.00 TBSP x_____
278	Orange Juice	1.00 CUP x_____
1382	Muffin-English-Plain-Toasted	1.00 ITEM x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
3	Cheese-Cheddar-Cut Pieces	1.00 OUNCE x_____
5610	Juice-Apple-Frozen-Concentrate-Diluted	1.00 CUP x_____
138	Mayonnaise-Soybean-Commercial	1.00 TBSP x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____
235	Banana-Raw-Peeled	1.00 ITEM x_____

DINNER

2528	Meatless Burger-Soy-Millst	2 BURGERS x_____
489	Roll-Hamburger-Plain	2.00 ITEMS x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____

SNACKS/OTHER

4910	Bagel-plain-3.5”	1.00 ITEM x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
8906	Cookie-Sandwich-Choc/Cream Filling	4.00 ITEMS x_____
223	Apples-Raw-With Skin-2 ¾ inch diameter	1.00 ITEM x_____
5622	Juice-Grape-Frozen-Concentrate-Diluted	1.00 CUP x_____
3493	Cake Mix-Spice-Super Moist	43.00 g x_____
114	Margarine-Regular-Unspecified Oils-TBSP	1.00 TBSP x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 3

BREAKFAST

278	Orange Juice	1.00 CUP x_____
1392	Waffles-Frozen-Each	3.00 ITEMS x_____
4558	Syrup-Pancake	3.00 TBSP x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
551	Jelly-Regular	2.00 TBSP x_____
524	Peanut Butter-Smooth Type	2.00 TBSP x_____
7292	Cookie-Oatmeal	3.00 ITEMS x_____
5238	Milk-Fluid-Skim	1.00 CUP x_____

DINNER

7056	Mixed Vegetables-Frozen-Boiled	1.00 CUP x_____
1564	Pasta-Noodles-Egg-Cooked	1.00 CUP x_____
839	Sauce-Sweet/Sour-From Mix-Prepared	1.00 CUP x_____
2837	Tofu-Raw-Regular	2.50 CUPS x_____

SNACKS/OTHER

235	Banana-Raw-Peeled	1.00 ITEM x_____
1813	Milk-Soy-Fluid	1.50 CUPS x_____
76	Ice Cream-Vanilla-Hardened-10% Fat	1.50 CUPS x_____
10001	Just Whites-Deb El Foods	3.00 TSP x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 4 (AEROBIC EXERCISE DAY)

BREAKFAST

5534	Cereal-Corn Flakes-Kelloggs-CNF	2.00 CUPS x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
278	Orange Juice	1.00 CUP x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
121	Three-Bean Salad	16 oz. x_____
105	Butter	10 g (2 pats) x_____

DINNER

1826	Salad-Green Salad-Tossed	200 g x_____
5445	Salad Dressing-Italian-Regular	2.00 TBSP x_____
332	Bread-Italian	1.00 SLICE x_____
10005	Pasta & Sauce/Italian Cheese/Lipton	2.00 SERVINGS x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
573	Green Beans	1.00 CUP x_____

SNACKS/OTHER

4911	Bagel-plain-toasted	1 ITEM x_____
4979	Jelly	1.00 TBSP x_____
105	Butter	5.00 g x_____
417	Cookie-Fig Bar	4.00 ITEMS x_____
223	Apples-Raw-With Skin-2 ¾ inch diameter	1.00 ITEM x_____

_____

POST AEROBIC EXERCISE SNACK-LOW CARBOHYDRATE –EAT IN EVENING AFTER EXERCISE

523	Peanuts	0.25 CUP (36 g) x_____
5221	Egg-Hard Boiled	140 g x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 5 (TEST DAY – ECCENTRIC EXERCISE + MUSCLE BIOPSY)

THIS DAY HAS BEEN ADJUSTED FOR THE PLACEBO BEVERAGE GROUP

BREAKFAST

5230	Egg-Chicken-Whole Scramble-Milk/Margarine	1.50 CUPS x_____
105	Butter	10 g (2 pats) x_____
551	Jelly-Regular	2.00 TBSP x_____
278	Orange Juice	1.00 CUP x_____
1382	Muffin-English-Plain-Toasted	1.00 ITEM x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
3	Cheese-Cheddar-Cut Pieces	1.00 OUNCE x_____
5610	Juice-Apple-Frozen-Concentrate-Diluted	1.00 CUP x_____
138	Mayonnaise-Soybean-Commercial	1.00 TBSP x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____
235	Banana-Raw-Peeled	1.00 ITEM x_____

DINNER

2528	Meatless Burger-Soy-Millst	2 BURGERS x_____
489	Roll-Hamburger-Plain	2.00 ITEMS x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____

SNACKS/OTHER

4910	Bagel-plain-3.5”	1.00 ITEM x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
8906	Cookie-Sandwich-Choc/Cream Filling	4.00 ITEMS x_____
223	Apples-Raw-With Skin-2 ¾ inch diameter	1.00 ITEM x_____
5622	Juice-Grape-Frozen-Concentrate-Diluted	1.00 CUP x_____
3493	Cake Mix-Spice-Super Moist	43.00 g x_____
114	Margarine-Regular-Unspecified Oils-TBSP	1.00 TBSP x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 5 (TEST DAY – ECCENTRIC EXERCISE + MUSCLE BIOPSY)

THIS DAY HAS BEEN ADJUSTED FOR THE MILK BEVERAGE GROUP

NO BREAKFAST (TEST/BIOPSY/TEST BEVERAGE)

LUNCH (12 NOON)

8670	Bread-Wheat	2.00 SLICES x_____
3	Cheese-Cheddar-Cut Pieces	1.00 OUNCE x_____
5610	Juice-Apple-Frozen-Concentrate-Diluted	1.00 CUP x_____
138	Mayonnaise-Soybean-Commercial	1.00 TBSP x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____
235	Banana-Raw-Peeled	1.00 ITEM x_____

DINNER

2528	Meatless Burger-Soy-Millst	2 BURGERS x_____
489	Roll-Hamburger-Plain	2.00 ITEMS x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____
138	Mayonnaise-Soybean-Commercial	1.00 TBSP x_____
1826	Tossed Salad	200 g x_____
5445	Salad Dressing-Italian	2 TBSP x_____

SNACKS/OTHER

4910	Bagel-plain-3.5”	1.00 ITEM x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
8906	Cookie-Sandwich-Choc/Cream Filling	4.00 ITEMS x_____
223	Apples-Raw-With Skin-2 ¾ inch diameter	1.00 ITEM x_____
5622	Juice-Grape-Frozen-Concentrate-Diluted	1.00 CUP x_____
3493	Cake Mix-Spice-Super Moist	43.00 g x_____
114	Margarine-Regular-Unspecified Oils-TBSP	1.00 TBSP x_____
5230	Egg-Chicken-Whole Scramble-Milk/Margarine	1.50 CUPS x_____
105	Butter	10 g (2 pats) x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 5 (TEST DAY – ECCENTRIC EXERCISE + MUSCLE BIOPSY)

THIS DAY HAS BEEN ADJUSTED FOR THE CARBOHYDRATE BEVERAGE GROUP

NO BREAKFAST (TEST/BIOPSY/TEST BEVERAGE)

LUNCH (12 NOON)

8670	Bread-Wheat	2.00 SLICES x_____
3	Cheese-Cheddar-Cut Pieces	1.00 OUNCE x_____
5610	Juice-Apple-Frozen-Concentrate-Diluted	1.00 CUP x_____
138	Mayonnaise-Soybean-Commercial	1.00 TBSP x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____
235	Banana-Raw-Peeled	1.00 ITEM x_____

DINNER

2528	Meatless Burger-Soy-Millst	1.5 BURGERS x_____
489	Roll-Hamburger-Plain	2.00 ITEMS x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____
138	Mayonnaise-Soybean-Commercial	1.00 TBSP x_____
1826	Tossed Salad	200 g x_____
5445	Salad Dressing-Italian	2 TBSP x_____

SNACKS/OTHER

4910	Bagel-plain-3.5”	1.00 ITEM x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
8906	Cookie-Sandwich-Choc/Cream Filling	4.00 ITEMS x_____
223	Apples-Raw-With Skin-2 ¾ inch diameter	1.00 ITEM x_____
5622	Juice-Grape-Frozen-Concentrate-Diluted	1.00 CUP x_____
3493	Cake Mix-Spice-Super Moist	43.00 g x_____
114	Margarine-Regular-Unspecified Oils-TBSP	1.00 TBSP x_____
5230	Egg-Chicken-Whole Scramble-Milk/Margarine	1.50 CUPS x_____
105	Butter	10 g (2 pats) x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 6 (BIOPSY II)

BREAKFAST

278	Orange Juice	1.00 CUP x_____
1392	Waffles-Frozen-Each	3.00 ITEMS x_____
4558	Syrup-Pancake	3.00 TBSP x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
551	Jelly-Regular	2.00 TBSP x_____
524	Peanut Butter-Smooth Type	2.00 TBSP x_____
7292	Cookie-Oatmeal	3.00 ITEMS x_____
5238	Milk-Fluid-Skim	1.00 CUP x_____

DINNER

7056	Mixed Vegetables-Frozen-Boiled	1.00 CUP x_____
1564	Pasta-Noodles-Egg-Cooked	1.00 CUP x_____
839	Sauce-Sweet/Sour-From Mix-Prepared	1.00 CUP x_____
2837	Tofu-Raw-Regular	2.50 CUPS x_____

SNACKS/OTHER

235	Banana-Raw-Peeled	1.00 ITEM x_____
1813	Milk-Soy-Fluid	1.50 CUPS x_____
76	Ice Cream-Vanilla-Hardened-10% Fat	1.50 CUPS x_____
10001	Just Whites-Deb El Foods	3.00 TSP x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 7

BREAKFAST

5534	Cereal-Corn Flakes-Kelloggs-CNF	2.00 CUPS x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
278	Orange Juice	1.00 CUP x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
121	Three-Bean Salad	16 oz. x_____
105	Butter	10 g (2 pats) x_____

DINNER

1826	Salad-Green Salad-Tossed	200 g x_____
5445	Salad Dressing-Italian-Regular	2.00 TBSP x_____
332	Bread-Italian	1.00 SLICE x_____
10005	Pasta & Sauce/Italian Cheese/Lipton	2.00 SERVINGS x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
573	Green Beans	1.00 CUP x_____

SNACKS/OTHER

4911	Bagel-plain-toasted	1 ITEM x_____
4979	Jelly	1.00 TBSP x_____
105	Butter	5.00 g x_____
417	Cookie-Fig Bar	4.00 ITEMS x_____
223	Apples-Raw-With Skin-2 ¾ inch diameter	1.00 ITEM x_____
523	Peanuts	0.25 CUP (36 g) x_____
5221	Egg-Hard Boiled	140 g x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 8 (BIOPSY III)

BREAKFAST

5230	Egg-Chicken-Whole Scramble-Milk/Margarine	1.50 CUPS x_____
105	Butter	10 g (2 pats) x_____
551	Jelly-Regular	2.00 TBSP x_____
278	Orange Juice	1.00 CUP x_____
1382	Muffin-English-Plain-Toasted	1.00 ITEM x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
3	Cheese-Cheddar-Cut Pieces	1.00 OUNCE x_____
5610	Juice-Apple-Frozen-Concentrate-Diluted	1.00 CUP x_____
138	Mayonnaise-Soybean-Commercial	1.00 TBSP x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____
235	Banana-Raw-Peeled	1.00 ITEM x_____

DINNER

2528	Meatless Burger-Soy-Millst	2 BURGERS x_____
489	Roll-Hamburger-Plain	2.00 ITEMS x_____
6332	Tomato/Lettuce	1.00 OUNCE x_____

SNACKS/OTHER

4910	Bagel-plain-3.5”	1.00 ITEM x_____
51	Milk-2% Fat-Lowfat-Fluid	1.00 CUP x_____
8906	Cookie-Sandwich-Choc/Cream Filling	4.00 ITEMS x_____
223	Apples-Raw-With Skin-2 ¾ inch diameter	1.00 ITEM x_____
5622	Juice-Grape-Frozen-Concentrate-Diluted	1.00 CUP x_____
3493	Cake Mix-Spice-Super Moist	43.00 g x_____
114	Margarine-Regular-Unspecified Oils-TBSP	1.00 TBSP x_____

ENSURE

_____

_____

_____

Name:_____Date:_____

Please check off foods as you eat them during the day. The bottom empty lines are for recording Ensure.

FOODS FOR DAY 9

BREAKFAST

278	Orange Juice	1.00 CUP x_____
1392	Waffles-Frozen-Each	3.00 ITEMS x_____
4558	Syrup-Pancake	3.00 TBSP x_____

LUNCH

8670	Bread-Wheat	2.00 SLICES x_____
551	Jelly-Regular	2.00 TBSP x_____
524	Peanut Butter-Smooth Type	2.00 TBSP x_____
7292	Cookie-Oatmeal	3.00 ITEMS x_____
5238	Milk-Fluid-Skim	1.00 CUP x_____

DINNER

7056	Mixed Vegetables-Frozen-Boiled	1.00 CUP x_____
1564	Pasta-Noodles-Egg-Cooked	1.00 CUP x_____
839	Sauce-Sweet/Sour-From Mix-Prepared	1.00 CUP x_____
2837	Tofu-Raw-Regular	2.50 CUPS x_____

SNACKS/OTHER

235	Banana-Raw-Peeled	1.00 ITEM x_____
1813	Milk-Soy-Fluid	1.50 CUPS x_____
76	Ice Cream-Vanilla-Hardened-10% Fat	1.50 CUPS x_____
10001	Just Whites-Deb El Foods	3.00 TSP x_____

ENSURE

_____

_____

_____

THIS IS THE LAST DAY OF THE CONTROLLED DIET. THE LAST BLOOD DRAW IS TOMORROW MORNING.

Appendix J

Muscle Soreness Scale

Muscle Soreness Recording Form

Muscle Soreness Scale

1 No Soreness

2 Minimal Soreness

3 Light Soreness

4 Moderate Soreness

5 Somewhat Strong Soreness

6 Strong Soreness

7 Very Strong Soreness

8

9 Very, Very Strong Soreness

10 Unbelievable Soreness

Muscle Soreness Rating

Subject:_____

Condition	Date	Numerical Soreness Rating
Prior to Eccentric Exercise (D5)		
Immediate post-eccentric		
3 h post-eccentric		
6 h post-eccentric		
24 h post-eccentric (D6)		
48 h post-eccentric (D7)		
72 h post-eccentric (D8)		
96 h post-eccentric (D9)		
110 h post-eccentric (D10)		

Muscle Soreness Scale:

1 No Soreness
2 Minimal Soreness
3 Light Soreness
4 Moderate Soreness
5 Somewhat Strong Soreness
6 Strong Soreness
7 Very Strong Soreness
8
9 Very, Very Strong Soreness
10 Unbelievable Soreness

Appendix K

Treatment Beverage Calculations

Treatment Beverage Calculations

Subject_____ **Beverage**: Milk CHO Placebo

Total kcal: _____kg bodyweight x 5 kcal/kg = _____kcal for treatment beverage

For Milk Beverages : 11g CHO 11 g Quik, skim milk 12 g CHO, 8 g pro, 236 ml = 8 fl. oz.

11g Quik + 236 ml milk = 23 g CHO, 8 g pro	124 kcal
22 g Quik + 118 ml milk = 46 g CHO, 16 g pro	248 kcal
5.5 g Quik + 59 ml milk = 11.5 g CHO, 4 g pro	62 kcal
5.75 g Quik + 29.5 ml milk = 5.75 g CHO, 2 g pro	31 kcal
2.9 g Quik + 15 ml milk = 2.9 g CHO, 1 g pro	15.5 kcal

Amount of Quik needed: _____g

Amount of Skim milk:_____ml _____**kcal**

For CHO beverages:_____kcal/4 = _____g CHO

13% solution: 30 g CHO in 236 ml water

1 g drink powder = 0.92 g CHO, so
_____g CHO / 0.92 = _____**g sport drink powder**

Amount of water needed:

30 g CHO x _____g CHO for subject
236 ml water *x* ml water

x = _____**ml water**

For Placebo: Use same amount of water for placebo beverage:_____ml

Appendix L

Urine Volume Recording Sheet

Urine Volume Data Sheet

Subject:_____

Day	Date	Volume (ml)
3		
4		
5		
6		
7		
8		
9		

Appendix M

Muscle Biopsy Care Instructions

Instructions For Care of the Muscle Biopsy Area

Here are some instructions for you to take care of the muscle biopsy area so that it heals well and that chance of infection is minimized. We've also included what is normal and what is not normal as part of the healing process.

General Instructions

- Keep the pressure wrap on for 8 hours following the biopsy
- Keep the steri-strips and Band-Aid on for 3 days.
- It is OK to shower, but you will want to avoid taking a bath or using a hot tub or swimming pool. Change Band-Aids after showering
- You are encouraged to walk on that leg; there is no need to "baby" it
- Report back to Ms. Rinehart 3 days after the last biopsy so she can examine the site for proper healing
- Remember not to consume any pain-relief medications or vitamin/mineral supplements without checking with us first

Normal Reactions Following Muscle Biopsy

- Localized stiffness, soreness, or bruising feeling of a light to moderate intensity
- There may be soreness and weakness in the leg that is noticeable when you go down stairs. You will want to go slowly, lead with the opposite leg, and use the handrail--or just use an elevator if possible

Reactions Not Normal Following Muscle Biopsy

- intense, excruciating pain in the leg or in the area of the biopsy
- bleeding which does not stop
- intense redness in the area of the biopsy
- heat in the area of the biopsy
- presence of pus
- fever
- hives or other signs consistent with allergic reaction (ex., difficulty breathing)
- IF YOU EXPERIENCE ANY OF THE ABOVE, CONTACT US IMMEDIATELY. IN CASE OF EMERGENCY, DO NOT HESITATE TO GO TO THE EMERGENCY ROOM OR CALL 911

  Janet Wojcik: 552-2953, 231-4900
  Dr. Janet Rankin: 231-6355, 552-9017
  Ms. Janet Rinehart: 231-2667
  Dr. Duane Lagan: mornings: 231-5671, afternoons: 231-5983
  University Student Health Services: 231-6444

Appendix N

Summaries of Statistical Analyses

Table 5.

Repeated Measures Analysis of Variance for Serum Insulin on Day 5

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.96	0.0818
Subjects(Group)	23	(79.0949)	
		Within Subjects	
Time	3	9.14	0.0001
Group x Time	6	4.03	0.0021
Subjects(Group) x Time	69	(59.2546)	

Note. Values enclosed in parentheses represent mean square errors.

Table 6.

Repeated Measures Analysis of Variance for Serum Growth Hormone on Day 5

Source	df	F	Pr > F
	Between Subjects		
Group	2	1.01	0.1904
Subjects(Group)	23	(6.8276)	
	Within Subjects		
Time	3	10.94	0.0003
Group x Time	6	1.79	0.0829
Subjects(Group) x Time	69	(6.4449)	

Note. Values enclosed in parentheses represent mean square errors.

Table 7.

Repeated Measures Analysis of Variance for Serum Glucose on Day 5

Source	df	F	Pr > F
	Between Subjects		
Group	2	0.42	0.3299
Subjects(Group)	23	(0.9544)	
	Within Subjects		
Time	3	6.57	0.0003
Group x Time	6	2.95	0.0064
Subjects(Group) x Time	69	(0.1788)	

Note. Values enclosed in parentheses represent mean square errors.

Table 8.

Repeated Measures Analysis of Variance for Morning Serum Cortisol

Source	df	F	Pr > F
		Between Subjects	
Group	2	0.58	0.2842
Subjects(Group)	23	(10402.9504)	
		Within Subjects	
Time	6	10.16	0.0001
Group x Time	12	0.55	0.4246
Subjects(Group) x Time	138	(1356.0864)	

Note. Values enclosed in parentheses represent mean square errors.

Table 9.

Repeated Measures Analysis of Variance for Serum Cortisol on Day 5

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.26	0.1508
Subjects(Group)	23	(4774.4158)	
		Within Subjects	
Time	3	47.11	0.0001
Group x Time	6	0.83	0.2746
Subjects(Group) x Time	69	(1746.6000)	

Note. Values enclosed in parentheses represent mean square errors.

Table 10.

Repeated Measures Analysis of Variance for Morning Serum Testosterone

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.82	0.0951
Subjects(Group)	23	(5.6351)	
		Within Subjects	
Time	6	2.33	0.0185
Group x Time	12	1.23	0.1341
Subjects(Group) x Time	138	(0.3102)	

Note. Values enclosed in parentheses represent mean square errors.

Table 11.

Repeated Measures Analysis of Variance for Serum Testosterone on Day 5

Source	df	F	Pr > F
		Between Subjects	
Group	2	0.36	0.3496
Subjects(Group)	23	(1.9947)	
		Within Subjects	
Time	3	46.20	0.0001
Group x Time	6	5.72	0.0001
Subjects(Group) x Time	69	(0.3932)	

Note. Values enclosed in parentheses represent mean square errors.

Table 12.

Repeated Measures Analysis of Variance for Morning Serum Creatine Kinase (CK)

Source	df	F	Pr > F
		Between Subjects	
Group	2	0.35	0.3556
Subjects(Group)	23	(10587888.605)	
		Within Subjects	
Time	6	2.79	0.0513
Group x Time	12	0.47	0.3277
Subjects(Group) x Time	138	(2096748.8474)	

Note. Values enclosed in parentheses represent mean square errors.

Table 13.

Repeated Measures Analysis of Variance for Logarithmic Transformation of Morning Serum Creatine Kinase (CK)

Source	df	F	Pr > F
		Between Subjects	
Group	2	0.15	0.4293
Subjects(Group)	23	(1.3741)	
		Within Subjects	
Time	6	6.69	0.0029
Group x Time	12	1.11	0.1793
Subjects(Group) x Time	138	(0.1498)	

Note. Values enclosed in parentheses represent mean square errors.

Table 14.

Repeated Measures Analysis of Covariance for Morning Serum Creatine Kinase with Creatine Kinase (CK) on Day 5 Morning as Covariate

Source	df	F	Pr > F
		Between Subjects	
Covariate	1	0.15	0.351
Group	2	0.23	0.399
Subjects(Group)	22	(15434509.703)	
		Within Subjects	
Time	4	2.27	0.07
Covariate x Time	4	0.29	0.3177
Group x Time	4	0.30	0.3899
Subjects(Group) x Time	88	(2137671.6524)	

Note. Values enclosed in parentheses represent mean square errors.

Table 15.

Repeated Measures Analysis of Variance for Serum Creatine Kinase on Day 5

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.90	0.0862
Subjects(Group)	23	(51725.0303)	
		Within Subjects	
Time	3	18.86	0.0001
Group x Time	6	2.31	0.04
Subjects(Group) x Time	69	(596.0630)	

Note. Values enclosed in parentheses represent mean square errors.

Table 16.

Repeated Measures Analysis of Variance for Morning Muscle Soreness

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.11	0.1737
Subjects(Group)	23	(5.2084)	
		Within Subjects	
Time	6	25.73	0.0001
Group x Time	12	0.31	0.4769
Subjects(Group) x Time	138	(1.1326)	

Note. Values enclosed in parentheses represent mean square errors.

Table 17.

Repeated Measures Analysis of Variance for Muscle Soreness on Day 5

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.33	0.1417
Subjects(Group)	23	(5.1806)	
		Within Subjects	
Time	3	18.79	0.0001
Group x Time	6	0.66	0.3283
Subjects(Group) x Time	69	(1.4926)	

Note. Values enclosed in parentheses represent mean square errors.

Table 18.

Repeated Measures Analysis of Variance for Isokinetic Peak Torque

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.91	0.855
Subjects(Group)	23	(10357.6623)	
		Within Subjects	
Time	3	31.70	0.0001
Group x Time	6	1.40	0.1275
Subjects(Group) x Time	69	(631.7347)	

Note. Values enclosed in parentheses represent mean square errors.

Table 19.

Repeated Measures Analysis of Variance for Isokinetic Max Repetition Work

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.36	0.1385
Subjects(Group)	23	(9578.2628)	
		Within Subjects	
Time	3	23.94	0.0001
Group x Time	6	1.69	0.0726
Subjects(Group) x Time	69	(607.3059)	

Note. Values enclosed in parentheses represent mean square errors.

Table 20.

Repeated Measures Analysis of Variance for Isokinetic Total Work for Five Repetitions

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.49	0.1227
Subjects(Group)	23	(217518.6473)	
		Within Subjects	
Time	3	30.03	0.0001
Group x Time	6	1.42	0.1195
Subjects(Group) x Time	69	(11446.0418)	

Note. Values enclosed in parentheses represent mean square errors.

Table 21.

Results of Repeated Measures Analysis of Covariance for Morning Body Weight Days 1-10 with Body Weight on Day 1 as Covariate

Source	df	F	Pr > F
		Between Subjects	
Covariate	1	14099.83	0.0001
Group	2	0.97	0.1982
Subjects(Group)	2	(2.0011)	
		Within Subjects	
Time	8	0.35	0.4611
Covariate x Time	8	0.69	0.3382
Group x Time	16	0.62	0.4193
Subjects(Group) x Time	176	(0.1976)	

Note. Values enclosed in parentheses represent mean square errors.

Table 22.

Repeated Measures Analysis of Variance for Skeletal Muscle Glycogen Measured on Experimental Days 5, 6, and 8

Source	df	F	Pr > F
		Between Subjects	
Group	2	0.41	0.3305
Subjects(Group)	23	(989.9012)	
		Within Subjects	
Time	3	97.00	0.0001
Group x Time	6	0.34	0.4103
Subjects(Group) x Time	69	(350.8081)	

Note. Values enclosed in parentheses represent mean square errors.

Table 23.

Results of Repeated Measures Analysis of Variance for Daily Urinary 3-Methylhistidine Excretion (μmol/day) Measured on Days 3-9

Source	df	F	Pr > F
		Between Subjects	
Group	2	0.81	0.2262
Subjects(Group)	23	(10911.6011)	
		Within Subjects	
Time	6	0.99	0.1036
Group x Time	12	0.83	0.2881
Subjects(Group) x Time	138	(2614.9938)	

Note. Values enclosed in parentheses represent mean square errors.

Table 24.

Results of Repeated Measures Analysis of Variance for Urinary 3-Methylhistidine Excretion Expressed Relative to Body Weight (μmol/kg/day) Measured on Days 3-9

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.61	0.1112
Subjects(Group)	23	(1.4726)	
		Within Subjects	
Time	6	1.30	0.1363
Group x Time	12	0.76	0.3312
Subjects(Group) x Time	138	(0.4305)	

Note. Values enclosed in parentheses represent mean square errors.

Table 25.

Results of Repeated Measures Analysis of Variance for Urinary 3-Methylhistidine Excretion Expressed Relative to Urinary Creatinine (μmol 3MH/ mmol creatinine) Measured on Days 3-9

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.62	0.1096
Subjects(Group)	23	(77.8436)	
		Within Subjects	
Time	6	1.74	0.069
Group x Time	12	0.56	0.411
Subjects(Group) x Time	138	(41.5741)	

Note. Values enclosed in parentheses represent mean square errors.

Table 26.

Results of Repeated Measures Analysis of Variance for Urinary 3-Methylhistidine Excretion Expressed Relative to Urinary Creatinine (μmol 3MH/ g creatinine) Measured on Days 3-9

Source	df	F	Pr > F
		Between Subjects	
Group	2	1.48	0.1239
Subjects(Group)	23	(5791.2490)	
		Within Subjects	
Time	6	1.71	0.0742
Group x Time	12	0.59	0.399
Subjects(Group) x Time	138	(3134.1409)	

Note. Values enclosed in parentheses represent mean square errors.

Table 27.

One-Way Analysis of Variance for Urinary 3-Methylhistidine Excretion (μmol/day) by Group on Day 5

Source	df	F	Pr > F
Between	2	3.43	0.0248
Within	23	(2192.8234)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Table 28.

One-Way Analysis of Variance for Relative Urinary 3-Methylhistidine Excretion (μmol/kg/day) by Group on Day 5

Source	df	F	Pr > F
Between	2	2.96	0.036
Within	23	(0.4660)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Table 29.

One-Way Analysis of Variance for Urinary 3-Methylhistidine Excretion Relative to Lean Body Weight by Group on Day 5

Source	df	F	Pr > F
Between	2	3.13	0.0314
Within	23	(0.65195791)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Table 30.

One-Way Analysis of Variance for Urinary 3-Methylhistidine Excretion/Urinary Creatinine Excretion (μmol 3MH/mmol creatinine) by Group on Day 5

Source	df	F	Pr > F
Between	2	0.02	0.49
Within	23	(67.9727)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Table 31.

One-Way Analysis of Variance for Urinary 3-Methylhistidine Excretion/Urinary Creatinine Excretion (μmol 3MH/g creatinine) by Group on Day 5

Source	df	F	Pr > F
Between	2	0.02	0.49
Within	23	(111.0946)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Table 32.

One-Way Analysis of Variance for Urinary 3-Methylhistidine (3MH) Excretion Relative to Lean Body Weight by Group on Day 5

Source	df	F	Pr > F
Between	2	3.13	0.0314
Within	23	(0.6519)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Table 33.

One-Way Analysis of Variance for Daily Carbohydrate Intake (%Energy) by Group

Source	df	F	Pr > F
Between	2	4.59	0.0210
Within	23	(0.2295)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Table 34.

One-Way Analysis of Variance for Daily Fat Intake (%Energy) by Beverage

Source	df	F	Pr > F
Between	2	3.64	0.0425
Within	23	(0.2255)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Table 35.

One-Way Analysis of Variance for Daily Energy Intake (Kilocalories) by Group

Source	df	F	Pr > F
Between	2	0.31	0.7352
Within	23	(1031186.3)	
Total	25		

Note. Values enclosed in parentheses represent mean square errors.

Appendix O

Raw Data

Table 36.

Raw Data For Serum Insulin on Day 5 (μIU/ml)

Group	Subject	AM	IPE	3H PE	6H PE
CP	1	11.1	8.1	18.1	22.3
	2	6.0	5.5	14.8	5.0
	5	6.2	5.0	10.7	5.2
	7	5.2	6.7	11.1	8.6
	8	6.4	5.0	23.6	19.0
	12	10.2	11.4	21.8	12.3
	16	6.4	7.6	45.1	12.2
	28	7.2	6.3	51.6	13.6
C	6	8.5	8.5	11.0	58.3
	10	5.6	8.1	7.7	6.5
	14	5.0	6.3	44.7	30
	17	5.6	8.1	13.8	6.8
	18	5.6	5.9	19.5	13.0
	19	8.8	5.4	12.0	18.6
	21	5.6	5.0	12.9	11.0
	25	5.0	5.0	14.6	6.2
	27	9.8	13.6	18.7	8.8
P	3	10.3	8.1	5.7	21.4
	4	5.0	6.6	5.0	5.0
	11	6.9	5.7	5.0	22.6
	13	6.0	7.3	6.1	25.8
	15	5.0	5.0	5.0	11.3
	20	9.0	6.8	5.0	6.6
	22	7.4	6.3	5.4	16.5
	23	10.7	12.5	5.0	11.6
	24	10.8	8.8	5.8	12.3

Note: AM, morning fasted value; IPE, immediate post exercise;
3H PE, 3 hr post-exercise; 6H PE, 6 hr post-exercise. C, Carbohydrate;
CP, Carbohydrate-Protein; P, Placebo.

Table 37.

Raw Data For Serum Growth Hormone on Day 5 (ng/ml)

Group	Subject	AM	IPE	3H PE	6H PE
CP	1	1.4	1.5	1.4	1.4
	2	1.4	1.4	1.4	1.4
	5	1.4	1.4	1.4	4.2
	7	1.4	2.3	1.4	1.4
	8	1.4	4.6	1.4	1.4
	12	1.6	2.6	1.4	7.8
	16	1.4	1.4	1.4	1.4
	28	1.4	5.0	1.4	1.4
C	6	1.5	2.8	1.4	1.4
	10	1.4	2.0	1.4	1.4
	14	1.4	1.4	1.4	1.4
	17	1.4	15.9	1.4	1.4
	18	9.3	6.8	1.4	1.4
	19	1.4	1.4	1.4	1.4
	21	1.4	13.2	1.4	1.4
	25	1.4	1.5	1.4	1.4
	27	1.4	5.0	1.4	1.4
P	3	1.4	3.2	2.3	1.4
	4	1.4	9.7	1.4	1.4
	11	1.4	14.5	1.4	1.4
	13	1.7	9.4	1.4	1.4
	15	1.8	13.6	3.1	1.4
	20	1.4	1.7	1.4	1.4
	22	1.4	1.4	1.8	1.4
	23	1.4	1.4	2.5	1.4
	24	1.4	1.4	1.4	1.4

Note: AM, morning fasted value; IPE, immediate post exercise;
3H PE, 3 hr post-exercise; 6H PE, 6 hr post-exercise. C, Carbohydrate;
CP, Carbohydrate-Protein; P, Placebo.

Table 38.

Raw Data For Serum Glucose on Day 5 (mmol/L)

Group	Subject	AM	IPE	3H PE	6H PE
CP	1	4.96	5.26	4.19	5.20
	2	5.56	5.56	5.43	5.25
	5	4.30	4.25	4.25	4.70
	7	4.11	4.15	4.03	3.79
	8	4.15	3.85	3.42	4.21
	12	4.87	5.86	5.50	5.38
	16	4.58	4.53	4.92	4.81
	28	4.78	4.78	6.16	5.44
C	6	5.02	4.87	3.95	5.85
	10	4.58	5.34	4.41	5.13
	14	5.10	5.43	5.82	6.87
	17	4.99	5.18	3.95	4.21
	18	4.30	4.87	4.59	5.22
	19	4.49	4.60	3.93	6.22
	21	4.56	4.73	4.35	5.20
	25	5.18	4.66	3.65	5.28
	27	4.29	4.47	6.16	5.44
P	3	4.59	4.59	5.02	5.38
	4	4.84	4.05	4.23	4.11
	11	4.73	5.63	4.55	4.97
	13	4.85	4.96	5.14	5.98
	15	3.84	4.36	4.24	4.30
	20	3.81	3.55	4.59	3.78
	22	4.21	5.02	4.21	3.92
	23	4.54	4.61	4.61	5.20
	24	4.48	4.74	5.24	5.43

Note: AM, morning fasted value; IPE, immediate post exercise;
3H PE, 3 hr post-exercise; 6H PE, 6 hr post-exercise. C, Carbohydrate;
CP, Carbohydrate-Protein; P, Placebo.

Table 39.

Raw Data For Serum Cortisol on Day 5 (ng/ml)

Group	Subject	AM	IPE	3H PE	6H PE
CP	1	126.3	202.9	78.6	60.6
	2	211.4	168.6	115.6	98.6
	5	244.8	254.8	51.8	56.6
	7	221.2	240.1	139.8	72.0
	8	150.2	152.6	54.7	60.1
	12	338.3	258.3	190.9	76.1
	16	221.6	212.8	133.4	74.6
	28	130.7	159.6	56.0	128
C	6	230.6	179.5	174.5	105.9
	10	236.4	243.6	138.4	92.3
	14	169.9	102.6	88.7	84.3
	17	144.7	239.6	65.9	48.3
	18	119.0	115.4	106.8	82.7
	19	131.8	124.3	79.0	164.2
	21	126.2	178.7	41.6	77.9
	25	165.3	131.9	84.7	92.7
	27	173.3	188.2	65.5	36.9
P	3	177.8	221.0	137.9	60.6
	4	138.9	166.1	94.8	65.7
	11	143.8	89.1	71.6	58.2
	13	143.5	192.5	59.7	66.7
	15	242.6	323.8	101.0	188.9
	20	214.6	233.9	99.1	91.3
	22	174.8	245.5	151.4	100.0
	23	257.8	292.9	89.9	87.4
	24	277.0	142.0	109.8	220.0

Note: AM, morning fasted value; IPE, immediate post exercise;
3H PE, 3 hr post-exercise; 6H PE, 6 hr post-exercise. C, Carbohydrate;
CP, Carbohydrate-Protein; P, Placebo.

Table 40.

Raw Data For Serum Testosterone on Day 5 (ng/ml)

Group	Subject	AM	IPE	3H PE	6H PE
CP	1	3.56	3.92	2.84	2.25
	2	7.06	7.44	3.96	3.63
	5	6.46	5.67	5.18	5.91
	7	5.51	6.14	5.06	3.51
	8	6.67	6.87	4.83	4.63
	12	6.77	6.75	4.39	4.75
	16	4.97	5.14	4.52	3.75
	28	6.63	5.86	5.06	3.28
C	6	3.57	3.87	3.82	2.97
	10	5.10	5.66	4.43	4.95
	14	5.98	7.20	5.42	3.64
	17	6.15	5.81	3.30	3.52
	18	5.27	5.24	4.06	2.84
	19	5.46	4.91	4.43	3.50
	21	6.66	6.86	5.31	4.33
	25	6.30	6.27	3.34	4.76
	27	6.55	6.56	3.93	4.06
P	3	3.79	5.10	4.74	3.68
	4	5.54	5.27	5.18	5.91
	11	5.47	4.68	5.49	3.25
	13	5.53	6.46	5.44	2.97
	15	4.66	5.17	4.52	3.75
	20	3.90	3.84	3.69	2.55
	22	4.45	4.72	4.61	3.58
	23	5.33	5.61	5.58	4.33
	24	5.05	3.75	5.70	3.98

Note: AM, morning fasted value; IPE, immediate post exercise;
3H PE, 3 hr post-exercise; 6H PE, 6 hr post-exercise. C, Carbohydrate;
CP, Carbohydrate-Protein; P, Placebo.

Table 41.

Raw Data For Serum Creatine Kinase on Day 5 (U/L)

Group	Subject	AM	IPE	3H PE	6H PE
CP	1	73.8	53.3	73.8	90.2
	2	51.3	53.3	61.5	108.7
	5	59.5	75.9	77.9	94.3
	7	86.1	90.2	90.2	92.3
	8	248.1	278.8	246.0	252.2
	12	41.0	36.9	39.0	59.5
	16	53.3	57.4	77.9	77.9
	28	69.7	73.8	65.6	80.0
C	6	444.9	477.7	473.6	543.2
	10	57.4	49.2	88.2	159.9
	14	49.2	67.7	47.2	67.7
	17	346.5	346.5	373.1	442.8
	18	387.5	354.7	377.2	406.0
	19	59.5	59.5	63.6	73.8
	21	55.4	47.2	102.5	123.0
	25	36.9	36.9	59.5	61.5
	27	51.3	45.1	206.9	248.1
P	3	43.1	47.2	61.5	84.1
	4	100.5	110.7	125.1	225.6
	11	153.8	164.0	149.7	190.7
	13	104.6	94.3	98.4	108.7
	15	209.1	217.3	211.2	282.9
	20	49.2	57.4	67.7	110.7
	22	80.0	86.1	92.3	82.0
	23	30.8	49.2	45.1	41.0
	24	57.4	51.3	67.7	55.7

Note: AM, morning fasted value; IPE, immediate post exercise;
3H PE, 3 hr post-exercise; 6H PE, 6 hr post-exercise.C, Carbohydrate;
CP, Carbohydrate-Protein; P, Placebo.

Table 42.

Raw Data for Muscle Soreness on Day 5 (1-10 Scale)

Group	Subject	AM	IPE	3H PE	6H PE
CP	1	1	2	2	3
	2	2	4	3	4
	5	4	6	6	6
	7	2	4	3	3
	8	2	3	5	5
	12	1	8	2	2
	16	1	1	2	2
	28	1	4	4	4
C	6	5	8	3	3
	10	1	5	4	4
	14	2	6	7	4
	17	1	4	1	1
	18	2	4	1	4
	19	1	3	3	4
	21	2	6	5	7
	25	1	4	1	1
	27	1	4	3	4
P	3	1	5	3	3
	4	1	1	2	3
	11	2	6	4	4
	13	1	1	1	1
	15	2	5	4	3
	20	1	1	3	3
	22	1	4	4	3
	23	1	5	3	1
	24	1	1	2	4

Note: AM, morning fasted value; IPE, immediate post exercise;
3H PE, 3 hr post-exercise; 6H PE, 6 hr post-exercise. C, Carbohydrate;
CP, Carbohydrate-Protein; P, Placebo.

Table 43.

Raw Data For Isokinetic Peak Torque for 5 Repetitions at 60^{o}/s (N*m)

Group	Subject	PRE	24H	48H	72H
CP	1	345.5	323.1	131.4	179.3
	2	187.4	151.3	168.7	143.6
	5	180.5	139.0	148.3	157.4
	7	190.0	170.6	151.3	141.7
	8	203.5	166.7	143.9	157.2
	12	227.8	155.8	131.1	161.4
	16	124.5	88.8	73.9	94.9
	28	204.8	170.0	152.6	163.5
C	6	172.0	166.1	143.9	157.2
	10	243.1	201.6	210.6	232.8
	14	155.0	71.1	90.0	118.8
	17	250.9	239.9	241.1	266.6
	18	217.1	206.2	205.3	204.1
	19	178.6	80.7	88.4	123.7
	21	254.3	218.3	213.2	182.9
	25	129.2	85.4	67.5	114.0
	27	265.1	226.5	217.9	243.9
P	3	246.8	79.5	56.7	59.3
	4	271.1	199.2	202.3	213.3
	11	283.8	250.2	257.9	263.5
	13	265.4	149.0	116.3	157.7
	15	269.2	239.2	246.7	264.8
	20	238.4	136.3	155.8	169.6
	22	214.2	181.4	176.3	179.4
	23	291.8	242.9	247.2	281.9
	24	286.7	236.4	233.1	258.0

Note: PRE, pre-experimental; 24H, 24 hr after eccentric resistance exercise;
48H, 48 hr after eccentric resistance exercise; 72H, 72 hr after eccentric resistance exercise.
C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 44.

Raw Data For Skeletal Muscle Glycogen (mmol/kg ww)

Group	Subject	24H	48H	72H
CP	1	36.0	77.9	64.1
	2	23.4	65.7	111.2
	5	45.6	95.5	89.8
	7	26.2	68.5	107.8
	8	24.2	56.4	111.3
	12	52.2	76.3	120.3
	16	37.5	51.2	136.1
	28	45.3	123.9	169.9
C	6	34.4	46.2	72.0
	10	15.8	38.7	84.7
	14	38.8	92.7	108.2
	17	46.8	100.2	94.9
	18	32.5	78.1	141.1
	19	29.9	69.7	58.0
	21	31.2	79.8	108.4
	25	36.2	104.6	123.5
	27	29.0	79.4	108.4
P	3	14.6	30.5	40.4
	4	38.0	72.7	84.6
	11	26.5	50.6	117.5
	13	41.0	81.7	119.9
	15	16.8	61.7	157.8
	20	17.3	66.6	69.8
	22	55.9	87.1	134.9
	23	28.1	69.7	68.9
	24	39.3	109.7	135.2

Note: PRE, pre-experimental; 24H, 24 hr after eccentric resistance exercise;
48H, 48 hr after eccentric resistance exercise; 72H, 72 hr after eccentric resistance exercise.
C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 45.

Raw Data For Total Energy (kcal) and %Energy Carbohydrate (CHO), Protein (Pro), and Fat.

Group	Subject	Kcal	%CHO	%Pro	%Fat
CP	1	3100	56	14	30
	2	2939	56	14	30
	5	2970	56	14	30
	7	2836	56	14	30
	8	3115	56	14	30
	12	3564	55	14	31
	16	3016	56	14	30
	28	2847	55	14	31
C	6	2844	55	14	31
	10	2762	56	14	30
	14	2752	55	14	31
	17	3543	56	14	30
	18	3059	55	14	31
	19	2939	56	14	30
	21	2964	55	14	31
	25	2835	56	14	30
	27	3004	56	14	30
P	3	2804	57	14	29
	4	2804	56	14	30
	11	2911	56	14	30
	13	3629	56	14	30
	15	4044	56	14	30
	20	2822	56	14	30
	22	2850	56	14	30
	23	2857	57	14	29
	24	2996	57	14	29

Note: C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 46.

Raw Data For Work Performed with the Maximal Repetition for 5 Repetitions at 60^{o}/s (N*m)

Group	Subject	PRE	24H	48H	72H
CP	1	278.8	202.6	119.7	164.2
	2	153.4	136.4	149.8	117.8
	5	191.0	148.2	158.4	145.5
	7	192.7	167.5	155.4	143.4
	8	207.5	168.4	179.3	200.4
	12	181.4	138.0	119.3	144.4
	16	124.6	84.8	73.9	95.6
	28	199.1	158.0	140.2	146.4
C	6	114.7	141.3	121.8	135.3
	10	242.7	199.3	214.0	212.3
	14	142.4	73.2	94.0	117.8
	17	270.3	262.9	269.3	283.9
	18	206.8	203.5	203.4	209.9
	19	168.1	83.4	99.9	132.8
	21	260.5	221.2	206.8	176.1
	25	129.2	87.5	77.2	117.4
	27	273.8	249.4	233.6	265.2
P	3	235.9	78.1	57.4	53.0
	4	235.4	191.3	115.7	198.5
	11	250.0	203.0	219.0	234.6
	13	255.2	146.6	122.9	243.7
	15	252.4	225.6	229.0	238.1
	20	225.4	145.4	158.7	175.6
	22	199.9	175.9	167.1	168.8
	23	272.4	220.4	210.3	252.5
	24	231.3	201.9	201.1	215.1

Note: PRE, pre-experimental; 24H, 24 hr after eccentric resistance exercise;
48H, 48 hr after eccentric resistance exercise; 72H, 72 hr after eccentric resistance exercise.
C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 47.

Raw Data For Total Isokinetic Work for 5 Repetitions at 60^{o}/s (N*m)

Group	Subject	PRE	24H	48H	72H
CP	1	1307.6	949.5	515.0	759.8
	2	716.8	620.2	364.4	561.7
	5	869.6	687.5	718.7	693.3
	7	877.5	807.1	727.5	647.2
	8	979.4	813.4	876.5	954.2
	12	847.5	628.7	540.1	686.6
	16	519.3	370.3	331.7	428.9
	28	975.4	748.2	675.2	705.5
C	6	657.0	648.3	541.5	640.6
	10	1125.6	887.0	946.9	992.0
	14	670.9	341.0	438.5	522.9
	17	1191.0	1212.0	1223.1	1262.7
	18	976.5	959.6	942.7	929.7
	19	779.4	394.5	446.5	610.7
	21	1236.4	1067.4	985.7	816.7
	25	612.4	401.6	361.6	552.0
	27	1326.2	1151.8	1102.2	1256.6
P	3	1077.3	361.3	251.8	235.5
	4	1115.4	900.1	931.8	941.2
	11	1219.0	965.6	1035.7	1084.8
	13	1218.5	678.8	554.6	729.5
	15	1200.5	1078.6	1103.9	1123.0
	20	957.9	652.0	734.3	814.0
	22	945.9	856.9	782.0	805.2
	23	1277.8	1030.0	963.3	1153.4
	24	1119.6	906.2	917.2	1024.3

Note: PRE, pre-experimental; 24H, 24 hr after eccentric resistance exercise;
48H, 48 hr after eccentric resistance exercise; 72H, 72 hr after eccentric resistance exercise.
C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 48.

Raw Data For Subject Characteristics.

Group	Subject	Age (yr)	Height (cm)	Weight (kg)	Body Fat (%)	VO_{2peak} (ml/kg/min)	1-RM (kg)	Physical Activity (min/week)
CP	1	28	186.5	114.8	21.2	36.7	109.1	0
	2	19	176.0	65.8	5.1	46.9	54.5	10
	5	21	180.0	75.3	8.9	45.1	56.8	120
	7	23	190.0	61.0	4.2	34.9	45.4	0
	8	67	170.0	66.3	11.6	45.6	50.0	420
	12	23	184.0	72.7	6.6	47.8	61.4	300
	16	19	168.5	67.1	7.0	40.7	38.6	240
	28	24	171.0	77.6	20.9	34.8	61.4	60
C	6	21	164.0	66.8	16.9	32.8	59.1	0
	10	22	182.5	74.1	7.0	43.1	66.2	240
	14	25	170.5	74.8	15.7	42.8	45.4	260
	17	29	177.5	76.1	5.1	48.1	77.3	345
	18	32	178.5	73.7	10.0	43.2	63.6	210
	19	23	178.5	69.4	5.7	41.5	52.3	375
	21	26	168.5	79.4	20.7	40.8	70.4	45
	25	27	167.0	55.5	5.1	46.3	52.3	120
	27	22	184.0	84.6	14.3	35.2	85.0	
P	3	20	177.5	93.1	28.2	32.8	63.6	0
	4	20	183.0	77.4	8.0	34.2	70.5	0
	11	24	182.5	79.8	13.1	42.8	63.4	300
	13	23	185.5	83.4	11.6	47.8	72.3	180
	15	29	168.5	73.4	14.5	48.0	68.2	0
	20	25	173.0	82.8	18.2	35.3	70.4	0
	22	25	170.5	65.3	6.6	44.5	59.1	45
	23	20	181.0	85.7	11.6	42.1	72.3	300
	24	21	166.0	68.0	6.1	34.8	61.4	0

Note: C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 49.

Raw Data For Morning Serum Cortisol on Experimental Days 4-9 (ng/ml)

Group	Subject	D 4	D 5	D 6	D 7	D 8	D 9	D 10
CP	1	203.6	126.3	199.1	201.0	139.8	137.4	202.0
	2	237.1	211.4	171.2	166.3	158.6	241.4	244.4
	5	203.8	244.4	100.2	161.6	200.4	193.7	164.3
	7	239.2	221.2	286.8	259.9	180.0	261.6	273.8
	8	168.6	150.3	115.4	86.5	148.5	147.7	168.4
	12	219.9	338.3	66.4	104.6	119.4	104.0	85.1
	16	197.4	221.6	155.7	127.8	118.8	168.8	206.8
	28	188.2	130.7	127.7	136.4	71.9	176.7	199.2
C	6	174.2	230.6	221.6	188.3	120.8	236.7	214.0
	10	218.4	236.4	241.2	227.7	218.8	244.1	235.4
	14	214.2	169.9	80.3	92.6	106.7	132.6	204.8
	17	159.1	144.7	147.8	169.2	143.6	178.7	141.2
	18	198.1	119.0	108.5	191.5	157.3	210.8	159.3
	19	111.3	131.8	141.8	97.9	157.0	103.5	128.6
	21	92.2	126.2	80.4	99.4	81.1	87.1	124.2
	25	138.7	165.3	107.7	141.7	120.6	233.9	174.3
	27	184.1	173.3	125.9	104.6	118.5	112.2	169.0
P	3	253.7	177.8	150.3	150.9	124.5	226.8	212.1
	4	179.2	138.9	83.4	123.9	113.5	183.1	137.4
	11	121.7	143.8	70.6	107.5	69.0	95.4	94.3
	13	77.5	143.5	165.9	157.2	132.9	133.3	152.3
	15	231.2	242.6	214.0	201.8	179.7	193.2	211.8
	20	235.5	214.6	145.2	134.8	110.7	217.2	237.9
	22	115.0	174.8	114.0	153.2	141.0	140.7	201.5
	23	234.9	257.8	191.4	220.4	192.5	216.7	257.3
	24	239.9	277.0	102.8	145.9	103.6	239.5	279.9

Note: Eccentric resistance exercise was performed on day 5. C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 50.

Raw Data For Morning Serum Testosterone on Experimental Days 4-9 (ng/ml)

Group	Subject	D 4	D 5	D 6	D 7	D 8	D 9	D 10
CP	1	4.74	3.56	4.03	5.40	3.46	4.80	4.45
	2	8.48	7.06	6.00	6.66	6.10	6.49	5.76
	5	6.29	6.46	5.48	4.88	5.55	5.49	50.5
	7	5.29	5.51	6.34	5.68	5.22	5.12	5.16
	8	7.42	6.67	7.72	7.06	5.66	5.53	6.91
	12	5.95	6.77	5.81	7.17	6.77	5.44	5.35
	16	5.78	4.97	5.02	5.25	5.20	5.10	5.01
	28	5.57	6.63	5.85	4.03	5.19	4.40	5.08
C	6	4.29	3.57	3.62	3.86	3.75	4.69	4.30
	10	5.77	5.10	5.36	4.70	5.19	5.42	5.40
	14	6.20	5.98	6.25	7.67	7.01	6.57	6.18
	17	6.67	6.15	7.01	7.77	6.49	5.91	6.17
	18	5.03	5.27	5.49	5.62	4.99	4.70	4.63
	19	5.95	5.46	5.17	4.15	4.65	6.01	6.35
	21	7.20	6.66	6.64	6.27	6.85	6.72	7.20
	25	8.13	6.30	6.48	6.77	6.77	7.57	7.31
	27	6.01	6.55	6.10	6.84	6.72	6.73	6.16
P	3	4.66	3.79	4.22	3.83	3.79	4.43	4.47
	4	6.88	5.54	5.65	5.12	6.26	6.23	5.68
	11	6.09	5.47	6.40	6.13	6.90	6.05	6.20
	13	5.38	5.53	4.44	4.91	5.28	4.02	4.67
	15	5.66	4.66	5.28	6.09	5.41	6.00	6.00
	20	3.48	3.90	3.58	3.76	3.38	4.20	2.98
	22	4.38	4.45	4.41	4.93	4.68	5.28	5.07
	23	5.53	5.33	5.53	5.20	5.33	4.86	4.83
	24	7.33	5.05	6.26	5.87	5.87	5.25	5.14

Note: Eccentric resistance exercise was performed on day 5. C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 51.

Raw Data For Morning Serum Creatine Kinase on Experimental Days 4-9 (U/L)

Group	Subject	D 4	D 5	D 6	D 7	D 8	D9	D 10
CP	1	59.5	73.8	1175	6306	12956	12628	9119
	2	47.2	51.3	94.3	82	57.4	55.4	43.1
	5	147.6	59.5	100.5	88.2	77.9	90.2	88.2
	7	80.0	86.1	82.0	96.4	80.0	104.6	114.8
	8	100.3	248.1	180.4	116.9	125.1	141.5	98.4
	12	41.0	36.9	53.0	69.7	51.4	41.0	14.4
	16	26.7	53.3	49.2	39.0	174.3	198.9	159.9
	28	41.0	69.7	59.5	39.0	28.7	28.7	116.9
C	6	88.2	444.9	393.6	227.6	237.6	209.1	116.9
	10	30.8	57.4	176.3	96.4	61.5	41.0	47.2
	14	32.8	49.2	51.3	57.4	34.9	39.0	34.9
	17	114.8	346.5	354.7	159.9	135.5	137.4	88.2
	18	264.5	387.5	293.2	145.6	88.2	55.4	55.4
	19	43.1	59.5	61.5	69.7	65.6	39.0	47.2
	21	34.9	55.4	94.3	110.7	1736.3	1578.5	1656.4
	25	32.8	36.9	49.2	45.1	49.2	39.0	55.4
	27	135.3	51.3	219.4	449.0	1198	1169	1148
P	3	24.6	43.1	172.2	2145	9086	7889	7249
	4	28.7	100.5	225.6	145.6	365.0	1241	1226
	11	100.5	153.8	186.6	143.5	121.0	133.1	131.2
	13	55.4	104.6	92.3	65.6	219.4	483.8	545.3
	15	84.0	209.1	367.0	299.2	881.5	1191	820.0
	20	28.7	49.2	73.8	110.7	215.3	375.2	371.1
	22	16.4	80.0	49.2	34.9	28.7	28.7	30.8
	23	32.8	30.8	59.5	39.0	102.5	73.8	121.0
	24	53.3	57.4	51.3	41.0	41.0	43.1	51.3

Note: Eccentric resistance exercise was performed on day 5. C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 52.

Raw Data For Morning Muscle Soreness Experimental Days 5-10 (1-10 Scale)

Group	Subject	D 5	D 6	D 7	D 8	D 9	D 10
CP	1	1	6	8	5	2	1
	2	2	5	4	5	4	5
	5	4	6	6	5	4	2
	7	2	3	3	3	2	1
	8	2	3	4	2	2	1
	12	1	6	4	2	1	1
	16	1	3	2	3	2	2
	28	1	2	2	2	1	1
C	6	5	3	4	2	2	1
	10	1	3	6	8	1	1
	14	2	4	3	2	2	1
	17	1	1	1	1	1	1
	18	2	3	4	3	2	2
	19	1	4	2	2	1	1
	21	2	7	4	4	2	1
	25	1	3	3	1	1	1
	27	1	2	4	3	2	2
P	3	1	5	7	7	3	3
	4	1	2	3	3	1	1
	11	2	2	3	1	1	1
	13	1	3	4	3	1	1
	15	2	2	4	4	3	2
	20	1	3	3	2	1	1
	22	1	3	3	1	1	1
	23	1	5	4	2	2	1
	24	1	4	3	2	1	1

Note: Eccentric resistance exercise was performed on day 5. C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 53.

Raw Data For 24 Hr Urinary Creatinine on Experimental Days 3-9 (g)

Group	Subject	D 3	D 4	D 5	D 6	D 7	D 8	D 9
CP	1	1.0	1.9	1.1	2.6	1.6	1.3	1.9
	2	1.5	1.5	0.5	2.0	1.8	1.0	1.0
	5	1.1	1.1	1.3	1.5	1.5	1.3	1.5
	7	0.8	0.7	0.9	1.1	1.1	1.0	1.2
	8	1.2	0.9	1.0	1.0	1.1	0.8	1.3
	12	1.3	1.5	1.4	1.2	1.3	0.6	1.5
	16	1.4	1.1	1.1	1.5	1.4	1.3	0.7
	28	0.7	1.1	3.0	0.6	0.9	3.0	0.8
C	6	1.6	2.5	2.0	1.9	2.1	2.1	1.8
	10	0.7	1.1	1.4	1.3	1.3	1.5	1.2
	14	1.1	1.1	0.9	0.9	0.9	0.8	0.9
	17	1.7	1.5	1.5	1.7	1.8	1.4	1.4
	18	1.1	1.2	1.5	1.4	1.4	1.4	1.1
	19	1.7	1.7	1.4	1.7	1.5	1.7	1.3
	21	1.4	1.7	1.8	1.7	1.3	1.7	1.6
	25	2.5	0.6	1.3	1.3	1.9	1.2	1.2
	27	1.4	3.4	1.0	3.3	1.5	1.6	1.8
P	3	0.9	1.8	1.0	1.4	1.2	1.4	1.0
	4	1.3	1.4	1.9	2.0	2.0	1.6	1.4
	11	1.2	1.3	1.7	1.1	1.7	1.6	1.7
	13	2.0	2.2	1.6	2.2	1.4	1.9	2.1
	15	2.2	1.6	1.6	1.3	1.2	2.1	1.7
	20	1.3	1.5	1.8	1.8	1.5	1.7	1.4
	22	1.4	1.4	1.3	2.1	1.1	1.4	2.3
	23	2.9	2.0	0.7	5.7	1.0	1.3	1.0
	24	1.9	1.5	1.0	1.1	2.3	1.7	1.5

Note: Eccentric resistance exercise was performed on day 5. C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 54.

Raw Data For 24 Hr Urinary 3-Methylhistidine Excretion on Experimental Days 4-9 (μmol/day)

Group	Subject	D 3	D 4	D 5	D 6	D 7	D 8	D 9
CP	1	377.1	299.2	231.8	310.5	310.9	308.9	339.6
	2	202.9	163.4	170.7	194.3	207.2	211.0	151.7
	5	117.4	121.4	165.5	203.6	144.0	94.1	137.4
	7	111.2	96.5	158.8	180.2	142.9	164.7	167.9
	8	204.9	221.6	244.2	150.3	185.6	229.7	215.3
	12	182.4	212.3	237.0	208.6	200.1	114.3	210.2
	16	196.8	144.1	189.3	246.1	220.4	169.3	90.3
	28	177.3	144.3	146.7	209.1	198.2	174.7	206.4
C	6	151.2	171.2	385.1	212.3	177.7	234.6	183.9
	10	135.1	212.8	230.1	203.2	191.1	220.4	165.4
	14	203.5	181.7	189.6	211.9	165.7	166.5	176.5
	17	238.4	178.1	247.0	184.6	198.0	173.7	196.3
	18	194.3	215.9	274.7	242.8	266.9	257.5	229.5
	19	200.3	209.3	156.6	250.3	212.5	205.8	151.9
	21	198.2	247.6	268.0	267.0	190.2	232.5	205.4
	25	185.0	183.5	206.8	180.2	166.7	188.3	198.5
	27	627.6	196.5	301.7	214.2	200.3	220.8	313.9
P	3	151.7	83.3	212.6	243.5	91.9	247.5	149.8
	4	120.6	158.0	243.8	224.6	184.3	161.3	131.8
	11	164.7	201.5	249.5	181.7	201.5	224.3	206.6
	13	208.8	248.9	218.5	239.3	201.1	218.4	241.6
	15	246.9	232.0	228.1	219.1	197.1	463.5	218.8
	20	147.8	198.0	222.9	216.3	181.1	186.7	170.2
	22	192.2	211.2	197.4	263.0	214.6	225.3	228.4
	23	277.6	219.6	264.2	129.7	213.0	161.0	272.4
	24	260.8	240.2	249.3	230.9	229.1	204.4	227.9

Note: Eccentric resistance exercise was performed on day 5. C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 55.

Raw Data For Morning Body Weight on Experimental Days 1-9 (kg)

Group	Subject	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10
CP	1	115.5	115.2	114.4	113.8	114.5	114.4	114.5	114.2	113.2	114.0
	2	65.3	65.5	64.7	64.8	64.7	64.5	64.2	64.7	65.2	64.9
	5	74.0	74.2	74.1	74.5	74.1	73.5	73.5	74.3	74.3	74.2
	7	62.0	62.5	62.4	62.3	61.6	62.7	62.2	63.2	63.2	62.8
	8	66.3	66.0	66.2	65.3	65.5	66.1	66.7	65.3	65.3	65.3
	12	72.6	71.3	71.9	71.7	71.5	72.4	72.5	72.7	72.0	72.0
	16	65.9	66.3	66.3	66.4	66.4	65.5	66.0	66.0	66.7	66.6
	28	79.0	78.3	78.0	77.4	77.5	77.7	77.7	77.5	77.8	77.4
C	6	69.1	70.0	69.4	69.5	69.1	68.7	68.3	69.0	70.0	69.5
	10	73.1	73.3	73.1	73.2	73.0	73.3	73.4	73.5	72.9	73.1
	14	75.6	75.9	75.8	75.7	75.7	75.7	75.8	76.1	75.7	75.5
	17	76.0	76.5	75.7	75.7	75.3	75.5	75.8	76.0	75.5	75.7
	18	73.7	73.7	73.5	73.0	72.8	72.8	72.8	72.8	72.9	72.8
	19	69.6	69.7	69.4	69.1	69.1	69.1	68.9	69.5	68.5	68.9
	21	80.0	79.7	79.6	80.0	79.7	79.7	79.9	79.7	79.5	79.5
	25	55.7	55.6	55.0	55.8	56.1	56.1	55.8	55.7	55.2	54.8
	27	86.2	86.3	85.4	85.0	85.3	85.3	85.7	85.5	85.7	85.5
P	3	93.7	93.4	93.0	93.6	92.8	92.8	93.2	92.8	93.5	93.7
	4	77.9	78.5	78.5	78.0	77.8	78.6	77.8	78.0	77.9	78.0
	11	79.7	79.9	80.5	79.9	79.8	79.3	79.5	79.5	78.9	78.9
	13	83.0	83.2	82.7	82.9	82.7	82.7	82.5	82.7	83.0	83.2
	15	73.0	73.6	73.2	73.8	73.2	73.7	73.8	72.9	73.2	73.8
	20	83.2	83.2	82.7	82.5	82.7	82.6	82.2	82.7	83.0	82.8
	22	64.5	64.8	64.9	64.1	64.7	64.9	64.7	64.7	64.7	64.4
	23	86.2	87.5	86.9	86.2	85.9	85.8	85.9	86.5	85.8	85.6
	24	69.8	70.0	68.7	68.7	68.6	68.6	68.7	69.4	68.9	69.1

Note: Eccentric resistance exercise was performed on day 5. C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 56.

Raw Data For Heart Rate (beats/min) During VO_{2peak} Testing

		Flywheel Resistance (kg)											Time to Peak
Group	Subject	Rest	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	(min)
CP	1	85	107	103	107	125	135	140	156	164	176	189	20:08
	2	76	86	100	114	130	144	160	170	181	187		17:00
	5	59	67	82	90	101	115	132	144	163	178		18:00
	7	64	83	103	114	126	141	150					13:20
	8	74	94	109	124	132	155	168	182	185			15:00
	12	82	99	109	124	134	144	162	173	186	197	203	19:18
	16	74	102	107	116	131	147	165	188	189			16:00
	28	87	99	108	126	139	155	170	190				13:00
C	6	76	93	105	115	129	147	167					11:55
	10	88	85	93	103	125	142	153	168	176			15:00
	14	73	82	93	115	129	144	162	166	180			18:00
	17	94	104	104	125	122	136	158	171	181	188	191	19:00
	18	74	90	110	119	136	142	154	167	177	188		17:00
	19	62	81	89	109	126	141	162	180	190	196		17:40
	21	64	93	109	133	142	162	173	183	196	207		17:00
	25	95	103	107	113	130	143	162	175	180			15:36
	27	69	90	101	114	132	147	157	176				14:00
P	3	74	72	99	112	130	139	160	175	182			16:00
	4	80	86	95	111	122	137	148	162	173			17:00
	11	101	101	116	129	136	167	170	186	196			17:52
	13	108	108	124	131	145	154	167	180	187	195	201	19:00
	15	89	90	98	105	116	129	144	159	175	181		18:00
	20	97	108	126	144	158	167	188	185	185			15:00
	22	75	105	116	126	147	160	165	173	184			16:15
	23	75	88	95	105	117	128	152	168	184	190		18:00
	24	87	115	127	152	165	179	189					11:09

Note: C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 57.

Raw Data For Overall/Legs Rating of Perceived Exertion (RPE, 6-20 scale) During VO_{2peak} Testing

		Flywheel Resistance (kg)											
Group	Subject	Rest	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	Peak
	1	6/6	7/7	7/7	9/9	9/9	10/10	11/11	13/13	13/14	16/16	18/18	18/19
	2	6/6	7/6	7/6	9/7	9/8	11/10	13/12	16/16				18/20
	5	6/6	7/6	7/6	8/6	10/9	11/10	11/11	13/13	18/19	20/20		20/20
	7	6/6	6/6	6/6	6/8	7/10	8/16	10/20					10/20
	8	7/6	7/7	7/8	8/10	10/13	13/14	15/17	16/19				16/19
	12	6/6	6/6	6/7	6/7	7/8	8/8	9/10	11/11	12/13	15/16		16/17
	16	6/6	6/6	6/6	6/6	7/7	8/7	8/8	11/11	14/14			14/14
	28	6/6	6/6	6/6	7/7	11/12	12/14	15/15					15/18
C	6	7/11	7/11	12/13	14/14	16/17	18/19	19/20					19/20
	10	6/6	6/6	6/8	9/10	12/13	15/17	17/18	18/19				18/19
	14	6/7	8/8	8/8	10/10	12/13	14/15	16/17	18/19	20/20	20/20		20/20
	17	6/6	6/6	6/6	6/6	6/7	6/6	6/8	8/10	11/15	16/18		16/18
	18	6/6	7/7	9/9	10/10	11/11	12/13	13/14	15/16	17/18	17/18		17/18
	19	6/6	7/8	7/8	12/13	13/14	14/16	15/17	17/19	20/20			20/20
	21	6/6	6/6	6/6	6/6	7/7	9/9	10/11	13/14	18/19	20/20		20/20
	25	6/6	6/6	7/6	6/6	6/8	8/9	8/12	13/15	15/18			15/19
	27	6/7	8/7	10/10	12/13	15/14	15/15	16/17	19/19				19/19
P	3	6/6	7/7	7/7	10/11	12/13	13/15	15/17	17/19	19/20			19/20
	4	6/6	6/6	7/7	9/8	9/10	10/12	13/14	16/18	17/19			17/19
	11	6/7	7/7	8/8	10/11	12/13	12/13	13/14	15/16	17/19			17/19
	13	6/6	6/6	6/6	6/7	6/9	8/10	8/10	11/12	15/16	17/19		17/19
	15	6/6	6/6	8/8	9/10	10/11	11/11	13/14	15/16	18/18	20/20		20/20
	20	6/6	6/6	7/7	7/8	8/10	11/13	15/17	20/20				20/20
	22	6/6	6/6	6/6	6/6	6/8	8/10	12/13	16/16	19/19			19/19
	23	6/6	9/9	11/11	12/12	13/13	15/15	17/17	20/20	20/20	20/20		20/20
	24	7/7	7/6	9/9	12/13	15/16	19/19	20/20	20/20				20/20

Note: C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 58.

Raw Data For Heart Rate (beats/min) During Endurance Cycling on Day 4

		Exercise Time (min)								Rest Taken
Group	Subject	5	10	15	20	25	30	35	40	(Y/N)
CP	1	162	162	162	168	180	180	186	186	Y
	2	162	162	168	168	173	174	174	180	Y
	5	127	130	144	144	147	132	142	140	Y
	7	150	159	162	163	165	150	157	157	Y
	8	143	157	161	166	170	173	156	166	Y
	12	183	190	191	196	190	191	182	183	N
	16	186	187	187	186	188	185	185	185	N
	28	169	181	185	191	187	191	196	200	Y
C	6	146	159	160	165	167	172	167	163	Y
	10	145	154	151	160	155	151	159	165	Y
	14	153	159	157	166	169	169	165	164	N
	17	150	161	166	169	175	172	167	167	N
	18	153	158	160	165	162	164	168	164	N
	19	158	168	162	167	165	175	171	171	N
	21	170	172	169	173	181	183	187	189	N
	25	156	175	175	182	180	185	189	188	N
	27	171	156	156	170	156	165	168	162	Y
P	3	162	162	168	173	173	180	180	180	Y
	4	153	164	173	173	165	167	170	170	Y
	11	162	174	177	181	175	184	187	187	Y
	13	179	179	186	187	190	189	190	189	N
	15	162	164	164	165	163	163	165	164	N
	20	167	164	168	170	176	174	179	179	N
	22	164	167	169	173	175	179	176	175	N
	23	167	176	182	181	181	182	184	180	Y
	24	173	183	179	184	185	182	187	180	Y

Note: C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Table 59.

Raw Data For Overall/Legs Rating of Perceived Exertion (RPE, 6-20 scale) During Endurance Cycling on Day 4

		Exercise Time (min)							
Group	Subject	5	10	15	20	25	30	35	40
CP	1	12/12	14/14	16/16	16/17	16/17	17/18	17/18	18/19
	2	12/11	12/12	12/13	13/13	15/13	15/14	16/14	16/15
	5	9/12	12/12	12/16	13/17	14/17	16/18	12/18	12/18
	7	10/12	11/14	12/14	13/14	15/18	10/10	12/13	12/16
	8	9/11	10/13	11/14	13/15	15/18	115/19	11/13	13/16
	12	12/12	13/14	15/16	16/17	15/16	16/17	15/15	15/15
	16	10/9	12/12	13/13	13/13	14/14	15/16	16/16	17/16
	28	11/11	13/13	15/15	16/16	15/15	16/17	17/17	19/19
C	6	11/13	11/13	13/15	15/16	16/17	17/18	17/18	18/19
	10	10/13	12/13	13/15	15/15	15/15	13/13	13/13	13/14
	14	13/14	14/15	16/16	16/17	17/18	18/19	18/19	19/19
	17	8/8	8/9	8/10	11/12	11/12	12/13	12/13	12/14
	18	13/13	13/13	14/15	14/14	13/14	13/14	14/13	14/15
	19	12/12	12/12	10/13	12/14	12/15	12/15	12/16	13/15
	21	7/7	11/11	12/13	12/14	12/15	13/16	13/16	13/18
	25	9/10	10/11	12/13	14/14	16/16	17/17	17/17	17/17
	27	12/12	13/13	14/15	15/15	15/15	16/16	17/17	18/18
P	3	9/10	11/13	12/14	12/14	13/16	14/16	13/17	14/18
	4	11/11	12/13	13/14	15/15	15/15	15/16	17/18	19/19
	11	12/13	13/15	14/15	14/15	14/15	14/15	14/15	15/15
	13	11/11	13/13	13/14	16/17	15/15	15/15	15/16	16/16
	15	13/14	14/15	17/18	18/18	19/19	19/19	19/20	19/20
	20	8/8	10/11	12/12	13/14	16/17	18/18	18/18	18/19
	22	8/8	11/11	11/13	11/14	11/14	11/15	11/15	11/15
	23	13/13	13/13	13/13	15/15	15/15	15/15	17/17	17/17
	24	13/13	15/15	15/15	15/15	17/17	16/16	17/17	17/17

Note: C, Carbohydrate; CP, Carbohydrate-Protein; P, Placebo.

Vita

Janet Regina Wojcik

Janet Regina Wojcik, daughter of Regina M. and the late John W. Wojcik, was born June 3, 1963, in Buffalo, NY. She has always had an interest in health and medicine. In 1983, she received an Associate of Applied Science degree in Medical Assisting from Erie Community College in Buffalo, NY. For three years she worked as a medical assistant at The Children's Hospital of Buffalo Division of Pediatric Cardiology. During this time, she also worked part-time as a meeting leader for Weight Watchers™ and became increasingly interested in exercise training and optimal nutrition for exercise.

She left The Children's Hospital in 1986 to pursue further education in the Exercise Science program at the State University of New York at Buffalo. She graduated Magna Cum Laude in 1990 with a Bachelor of Science degree in Sport and Exercise Studies. Janet has held a variety of fitness instructor positions in corporate fitness centers, resorts, personal training studios, and community centers. In 1991, she obtained certification as a Health/Fitness Instructor™ from the American College of Sports Medicine.

In 1992, Janet received an opportunity to pursue graduate studies at the Canisius College of Buffalo, NY. She received a Master of Science in Physical Education in 1994. While at Canisius, she immensely enjoyed working as a teaching and research assistant in a scholarly environment and decided to pursue further education at the doctoral level.

On a personal note, Janet has additional loving family members consisting of 3 brothers, Robert, Jim, and Len; 1 sister, Margaret; and 10 nieces and nephews. In her spare time she enjoys exercising--outdoor cycling in particular, listening to rock-n-roll and blues, and is a Ford Mustang enthusiast.