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Impact of protein supplementation on muscle recovery after exercise-induced muscle soreness

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Impact of protein supplementation on muscle recovery after exercise-induced muscle soreness

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I dedicate this thesis to Dr. Paul Flakoll,

who inspired me to become more than I thought I could be.

Thank you, Dr. Flakoll

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ABSTRACT

We investigated whether protein (PRO [0.4g/kg]) vs. carbohydrate (CHO [0.4g/kg]) vs. placebo nutrition supplements would alleviate muscle soreness when consumed immediately after eccentric exercise in 21 untrained men aged 18-30 years. During this double-blind randomized block study design, each subject completed three, 3-day trials (separated by $\geq$2 weeks), identical except for treatment, with each serving as his own control. Trials began with a bout of right leg eccentric exercise (Biodex), followed directly by treatment. At 0 (baseline), 24, and 48 hours, data were collected: creatine phosphokinase (CPK) from pre-exercise blood samples, subjective muscle soreness questions, and strength tests (power, torque, work.). ANOVA indicated that exercise caused mild muscle damage, evidenced by an overall day effect ($p \leq 0.0001$) for muscle soreness, with the lowest median values (0 to 10 scale) on day 1 (0.7), increasing ($p \leq 0.0001$) on day 2 (3.2), and remaining elevated on day 3 (3.4). We also noted an overall day effect ($p \leq 0.0001$) for CPK, with lowest median values (U/L) on day 1 (136), increasing ($p \leq 0.0001$) on day 2 (235), and remaining elevated on day 3 (189). ANOVA revealed no significant treatment effect on indicators of soreness or damage during recovery. Our results indicated that a PRO or CHO supplement after exercise causing mild muscle damage did not facilitate muscle recovery in adequately nourished, healthy young men.

CHAPTER I: GENERAL INTRODUCTION

OBJECTIVE

The purpose of this study is to determine whether distinct nutritional supplements (protein vs. carbohydrate vs. placebo) cause different effects on muscle recovery after exercise-induced muscle soreness in untrained men aged 18 – 30 years.

HYPOTHESIS

1) A protein supplement will promote more rapid and greater muscle recovery after a single bout of exercise-induced muscle soreness compared with an isocaloric carbohydrate supplement.
2) A protein supplement will promote more rapid and greater muscle recovery after a single bout of exercise-induced muscle soreness compared with a non-isocaloric placebo.
3) A carbohydrate supplement will promote more rapid and greater muscle recovery after a single bout of exercise-induced muscle soreness compared with a non-isocaloric placebo.

SPECIFIC AIMS

1) To determine whether a protein supplement promotes more rapid and greater muscle recovery than carbohydrate after exercise-induced muscle soreness in untrained men aged 18 – 30 years by measuring plasma creatine phosphokinase (CPK) activity.
2) To determine whether a protein supplement promotes more rapid and greater muscle recovery than placebo after exercise-induced muscle soreness in untrained men aged 18 – 30 years by measuring plasma CPK activity.
3) To determine whether a carbohydrate supplement promotes more rapid and greater muscle recovery than placebo after exercise-induced muscle soreness in untrained men aged 18 – 30 years by measuring plasma CPK activity.

SIGNIFICANCE OF STUDY

Exercise-induced muscle soreness is thought to be caused by a mechanical disruption of sarcomeres, causing impaired excitation-contraction coupling and calcium signaling, followed by activation of calcium-sensitive degradation pathways. Eccentric exercise in

particular causes muscle soreness and microdamage and large increases in serum CPK activity (Evans 1991). Muscle damage is recognized by ultra-structural changes in muscle architecture, increased muscle proteins and enzymes (such as CPK) in the bloodstream, loss of muscular strength and range of motion, and muscle soreness (Evans 1991). In this study, some of these muscle damage characteristics will be recorded to track muscle soreness.

During exercise, energy is used for contraction and is directed away from protein synthesis. Amino acids are also directed away from protein synthesis and are instead used to form glucose for additional energy. After exercise, amino acids and/or energy may be limiting and thus prevent optimal muscle protein synthesis (Evans 1991; Ohtani et al 2006). Hence, a protein supplement immediately after exercise has considerable potential to decrease muscle damage and soreness. In previous studies, increases in whole body and muscle protein accretion were noted when a protein supplement was ingested immediately following exercise. Studies reported that amino acid supplements given after exercise led to quicker recovery from muscle fatigue, decreased plasma CPK activity, decreased muscle soreness, and prevented exercise-induced proteolysis (Ohtani et al 2006; Nissen et al 1996). Further research suggested that post-exercise protein ingestion is most beneficial to muscle protein accretion when consumed immediately after exercise versus several hours later.

Current dietary recommendations for athletes are similar to those for the general population, but they emphasize carbohydrate and fluid intake (ADA 2000). The purpose of this recommendation is not to decrease muscle soreness, but rather to ensure that sufficient glycogen stores are available for muscle use and to avoid dehydration. It is also recommended that athletes consume extra protein (1.2-1.7 g/kg body weight/day) to have adequate muscle-building "supplies" (ADA 2000). However, it is unknown which macronutrient will be most effective in decreasing muscle soreness and increasing muscle recovery when consumed immediately after exercise. It is unclear how the nutrient supply affects muscle soreness. Past studies examined the effects of nutrition on exercise; several reported that, when compared with carbohydrate-only supplements, protein/carbohydrate-containing supplements led to lower CPK activity, reduced muscle soreness, and improvements in time to fatigue (Wojcik et al 2001; Saunders et al 2004). More research is necessary, however, to fully understand the effects of protein and carbohydrate supplements

on muscle soreness. Therefore, this study compares the effects of protein, carbohydrate, and placebo on muscle recovery after a single bout of exercise-induced muscle soreness.

LIMITATIONS OF STUDY

The study did not include a random sample of participants, but rather a convenience sample of self-selected male volunteers between the ages of 18 and 30 years. Hence, the results may not be generalizable to the entire male population. Also, this study did not include females, and thus conclusions from the data cannot be drawn for females. In addition, this study evaluated a relatively small sample size of 21 people. A trial with a greater number of subjects would provide greater statistical power for detecting small changes, which may not have been detectable because of inherent inter-individual variability.

Another limitation is the extent to which we could monitor the subjects' physical activity. Although the subjects were instructed to refrain from physical activity for one week before each trial, their actual physical activity was not quantified. Thus, their physical activity may have had an undetectable effect on muscle soreness and CPK activity. Also, this study did not examine inflammatory markers that may result from or have had an effect on muscle damage. Although each subject's diet was discussed with him and monitored, dietary intake was not controlled *per se*, allowing for inter-individual variation. However, the intent of the study was to include free-living men.

The exercise bouts were performed on a Biodex machine, which uses a person's own resistance to determine the level of power output. Although subjects were strongly encouraged to work "as hard as they can", the work performed from subject to subject typically varies. However, because each person served as his own control, the subject's effort should not have been a major issue as long as each person performed similarly from trial to trial.

DEFINITIONS

Concentric action - a shortening of a muscle during its contraction

Delayed onset muscle soreness (DOMS) - muscle soreness or discomfort that appears 24 to 48 hours after exercise

Eccentric action - a lengthening of the muscle during its contraction; controls speed of movement caused by another force

Isokinetic exercise - exercise in which the rate of movement is constantly maintained through a specific range of motion although maximal force is exerted

Isometric exercise - an activity in which the muscles exert force but do not visibly change in length (i.e., pushing against a wall)

Isotonic exercise - an activity in which the muscles exert force and change in length as they lift and lower resistance (i.e., bicep curls)

Muscular strength - the ability of the muscle to generate the maximum amount of force

Level of exertion evaluation - scale of 0-10 that rates how much a person feels he/she is exerting during a particular exercise set

Peak torque - the maximal torque value that occurs in the range of movement

Power - the product of torque and angular velocity, or work divided by time

Range of motion - the angular displacement between the start and stop angles

Repetition - the number of times an exercise is repeated within a single exercise "set"

Set - a group of repetitions of a particular exercise

Work - calculated as a measure of strength when torque is the unit of measurement

CHAPTER II: REVIEW OF LITERATURE

INTRODUCTION

In general, unaccustomed exercise causes muscle damage. The sensation of muscle damage is often referred to as *delayed onset muscle soreness* (DOMS). DOMS usually begins within 24 hours and peaks within 48 hours after exercise. The severity of DOMS depends upon many factors, perhaps most prominently on exercise intensity and type and the training level of the individual (Evans 1991).

Two general types of exercise muscle action exist: concentric and eccentric actions. A concentric action occurs when a skeletal muscle contracts and shortens, whereas an eccentric action occurs when a skeletal muscle lengthens as it produces force (Evans 1991). For example, a concentric action is lifting a weight, and an eccentric action is lowering the weight (Evans 1991). Intense eccentric exercise typically causes extensive muscle damage, whereas an equal amount of concentric exercise may cause very little damage (Newham et al. 1983b). Compared with concentric actions, eccentric actions cause more severe muscle damage, DOMS (Newham et al. 1983b), and increased plasma creatine phosphokinase (CPK) activity (Gleeson et al. 1995). The difference in muscle damage is thought to be the result of different fiber recruitment patterns and the fact that greater tension per muscle fiber is generated under eccentric exercise conditions (Newham et al. 1983c). This greater force-to-fiber ratio causes greater muscle damage (Evans 1991).

HUMAN RESPONSE TO MUSCLE DAMAGE

Following exercise-induced muscle damage in humans, several responses are evident. However, the extent of damage is not immediately evident; muscle damage seems to peak one to three days after the exercise occurs (Newham et al. 1983b). This is demonstrated from muscle biopsies and increased CPK activity (Newham et al. 1983b). Common responses to exercise-induced muscle damage include increased CPK, DOMS, decreased peak torque, decreased power output, and decreased range of motion (Evans 1991).

Creatine Phosphokinase (CPK)

Perhaps the best-studied response to muscle damage is that of increased CPK activity, and thus CPK has been used as the most common indicator of skeletal muscle damage after muscular exercise:

$$\text{ADP + creatine phosphate} \xrightarrow{\text{CPK}} \text{ATP + creatine}$$

Eccentric exercise produces a much larger increase in CPK, as noted in a study (Newham et al. 1986) that compared uphill walking (concentric exercise) with downhill walking (eccentric exercise). This study found that the eccentric exercise (700-1500 IU/L) caused much greater CPK activity than did the concentric exercise (60-200 IU/L). Another similar study (Newham et al. 1983a) found that subjects participating in eccentric stepping exercise (up to 34,500 IU/L) showed a much greater CPK response than did subjects participating in concentric stepping (less than 400 IU/L). The response of CPK to exercise is extremely variable and is influenced by several factors, including exercise type, intensity, and the training level of individuals. However, it is also important to note that there is enormous inter-individual variability, with subjects who perform identical activities often showing vastly different CPK activity increases (Evans 1991). Although the exact reasons for this phenomenon are unknown, it is suspected by researchers that the large variability of the CPK response after exercise is related to the variability of exercise-induced muscle damage (Nosaka et al. 1996). Thus, it is important that each subject serves as his/her own control, thereby minimizing the effect of inter-individual variability in exercise-induced muscle damage.

Delayed-Onset Muscle Soreness

Another observed response, usually occurring from one to five days following exercise, is DOMS, often recorded as a feeling of muscle soreness, tenderness, or stiffness. The exact cellular mechanism causing DOMS is unknown, but two hypotheses include cell membrane damage leading to disruption of calcium homeostasis and/or structural damage from high tension in the contractile/elastic system (Armstrong et al. 1984). Eccentric exercise causes greater DOMS than concentric exercise; in fact, concentric exercise often results in no

detectable DOMS (Newham et al. 1983a). Intensity and duration of exercise are also important factors in DOMS. Although not dangerous in itself, DOMS may increase the risk of further injury if an individual exercises subsequently without allowing time for the muscle micro-damage to heal (Cheung et al. 2003). In addition, DOMS can cause decreased joint range of motion and peak torque, as well as alterations in muscle sequencing and recruitment patterns. This may cause unaccustomed stress to be placed on muscle ligaments and tendons (Cheung et al. 2003). Many treatments have been attempted to decrease DOMS, such as pharmaceuticals, herbal remedies, stretching, massage, and various nutrition supplements, but none have been demonstrated as consistently successful (Connolly et al. 2003). Light concentric physical activity does not seem to have an effect on the recovery from previous muscle damage caused by eccentric exercise, although it may have a temporary analgesic effect on DOMS (Zainuddin et al. 2006).

Other Responses

Decreased peak torque, decreased power output, and decreased range of motion are other consistent human responses to muscle damage, and they are commonly used in research studies to monitor muscle damage and recovery after exercise (Chapman et al. 2006; Vincent et al. 1997; Paschalis et al. 2005b).

PROTOCOL SELECTION

Many possibilities exist when designing a research protocol for muscle-damaging eccentric exercise. This section indicates some of the most effective protocols for inducing muscle damage in a research setting.

Eccentric Exercise Protocols

Several different eccentric exercise techniques may be used to incur muscle damage, such as running downhill (Newham et al. 1986), stepping down (Newham et al. 1983a), or action of the elbow flexors (Chapman et al. 2006) or knee extensors (Paschalis et al. 2005a) on a dynamometer. Each protocol has benefits and downfalls, whereas the magnitude of muscle damage varies among models. A study involving 11 young healthy untrained men examined indicators of muscle damage by comparing knee and elbow eccentric exercises using the same relative intensity of exercise (Jamurtas et al. 2005). The data found similar changes in DOMS, but changes in CPK and peak torque were larger from the arm compared

with leg exercises. The study concluded that the magnitude of muscle damage was greater and the recovery slower after exercising the elbow flexors verses the knee extensors (Jamurtas et al. 2005).

However, exercising the knee extensors may be the best choice, depending upon the intent of the research. Some studies desire only a moderate degree of exercise-induced muscle damage; this causes the study to be more similar to real life. Typically, eccentric exercise with the knee extensors is performed on a dynamometer, such as the Biodex isokinetic dynamometer, a machine created for physical therapy patients. A common protocol is for a person to use one leg to perform several sets of maximal voluntary repetitions at a particular angular velocity, with sets separated by a short rest period. For instance, one recent study (Paschalis 2005a) induced muscle damage eccentrically with 10 healthy male volunteers (age 22-24 years) who performed 120 (12x10) maximal voluntary repetitions on one leg at the angular velocity of 60 degrees/second (deg/s). Another study (Babul et al. 2003) had subjects perform 300 maximal voluntary eccentric contractions (30 sets of 10 repetitions per minute) on a dynamometer with one leg at the angular velocity of 30 deg/s. As these studies indicate, eccentric exercise with the knee extensors as described is a common and accepted practice.

Between sets of eccentric contractions, subjects are usually provided a short rest period to allow recovery from the previous bout. This rest increases the amount of peak torque that subjects can produce during the next set. The length of this rest period varies among studies, but a recent study (Parcell et al. 2002) reported that during a common isokinetic strength test protocol, a rest period of at least 60 seconds between sets was necessary for recovery before the next set to allow the subjects to produce their maximum peak torque. Also, faster velocities of exercise required longer rest periods to recover.

Fast vs. Slow Velocity Eccentric Exercise

It is currently debatable whether fast or slow velocity eccentric exercise causes greater muscle damage. One recent study (Chapman 2006) used 12 untrained subjects to perform a series of slow velocity isokinetic eccentric elbow flexions with one arm (30 deg/s) and fast velocity exercises on the other arm (210 deg/s), separated by 14 days. Using measures of CPK, maximal torque, and muscle soreness, this study found that fast velocity

eccentric exercise caused greater muscle damage than slow velocity exercise. Another very similar study (Paddon-Jones et al. 2005) found that muscle soreness severity was similar from two exercise velocities, but the fast exercises caused peak muscle soreness 48 hours later than the slow exercises. This study concluded that the velocity of eccentric exercises differentially influenced the magnitude and time course of muscle damage markers.

High vs. Low Intensity Eccentric Exercise

Another recent study (Paschalis et al. 2005b) compared the effects of equal volumes (equal total work performed) of high verses low intensity eccentric exercise on muscle damage. This study enrolled 12 untrained healthy young men to undergo two isokinetic quadriceps eccentric exercise sessions, one with each leg, separated by two weeks. Subjects first performed high intensity eccentric exercise consisting of 12 sets of 10 maximal voluntary efforts, followed by low intensity eccentric exercise performed continuously at 50% peak torque until the total work performed was equal to the high intensity exercise. Peak torque indicators, CPK activity, DOMS, and range of motion were measured at 0, 24, 48, 72, and 96 hours post-exercise. The study results indicated similar effects of high versus low intensity eccentric exercise on muscle damage, but high compared with low intensity exercise caused a larger decrease in muscle performance as measured by peak torque (Paschalis et al. 2005b).

Protective Effect of Eccentric Exercise

In sedentary subjects, only one bout of eccentric exercise can cause a "protective effect" on muscle damage that may last for several weeks or months. A low amount of eccentric exercise, causing no perceivable muscle damage or DOMS, is sufficient to cause a protective effect against, and significantly improve recovery from, further eccentric exercise (Paddon-Jones et al. 2001). One study (Byrnes et al. 1985) compared DOMS and CPK activity in three groups of subjects. All groups performed identical eccentric exercise, and then one of the groups repeated the same eccentric exercise 3, 6, or 9 weeks later. The results for the groups that exercised 3 or 6 weeks later indicated that subjects had significantly smaller DOMS scores and CPK activity from the second trial compared with the first trial. However, the group that exercised 9 weeks later showed no difference in DOMS or CPK activity between the two exercise sets. Thus, this study concluded that a single eccentric

exercise bout caused a protective effect, preventing repeated extensive damage from a similar bout for up to 6 weeks. Hence, after the initial exercise, subjects typically experience protection against further eccentric exercise, with smaller increases in DOMS and CPK activity. Further, another similar study (Nosaka et al. 1991) found that the CPK increases were significantly smaller after a second bout of eccentric exercise performed 6 months after the first bout; this research group subsequently demonstrated that the protective effect lasted between 9 and 12 months (Nosaka et al. 2001). Thus, the precise duration of the protective effect is currently unknown, as is the mechanism of this protection.

SUBJECT SELECTION

Male vs. Female Subjects

Subject selection for a study also presents many options, with previous research indicating the most appropriate subject sample to use. Males are superior subjects in studies measuring DOMS, because females' sensations of pain and soreness are affected by estrogen concentration, as well as males' inherent ability to create more torque, power, and work (Pincivero et al. 2003). A study examining the effect of estrogen on markers of muscle tissue damage found that an elevated estrogen concentration has a protective effect on muscle tissue following eccentric exercise, as demonstrated by lower CPK activity in the high-estrogen group of subjects (Carter et al. 2001). In contrast, another study found that women taking oral contraceptives (and thus having high circulating estrogen) had delayed strength recovery (as measured by maximal isometric strength) after eccentric exercise, as compared with women not taking oral contraceptives (Savage et al. 2002). Also, a study comparing gender-specific knee exercises found that during maximal effort muscle contractions, males were able to generate higher knee extensor and flexor torque, work, and power than were females, and males also showed a higher susceptibility to muscle fatigue (Pincivero et al. 2003).

Number of Subjects

Because of the large inter-subject variability of CPK activity, the necessary number of subjects needed for a study designed to determine eccentric exercise and its related effects is very difficult to ascertain based upon power studies. Examining these studies, subject numbers ranging from 5 to 20 is common. In addition, BMI ranging from 18 to 30 is also common (Zainuddin et al. 2006; Paschalis et al. 2005b; Eston et al. 1996; Nelson et al. 2004).

Trained vs. Untrained Subjects

An individual's previous training significantly affects the amount of muscle damage that results from eccentric exercise. One study (Vincent et al. 1997) compared trained individuals (at least 3 years of weightlifting experience) and untrained individuals (no regular resistance exercise for at least 3 years). After performing eccentric exercise on the legs, they measured the subjects' DOMS (using a 100 mm visual analog scale), CPK activity, and peak torque on the Biodex isokinetic dynamometer. The results indicated that although trained subjects actually reported greater DOMS, the CPK activity was significantly lower than that of the untrained subjects. These results suggest that trained subjects can develop extensive soreness without the same increase in CPK activity observed in untrained subjects.

PROTEIN METABOLISM DURING AND AFTER EXERCISE

Following eccentric exercise, many changes in metabolism occur. Exercise increases energy expenditure up to 10-fold, and thus recovery from muscle damage is almost certainly affected by nutrition (Fielding et al. 2002). Although several research studies indicate changes in both protein and carbohydrate metabolism, the optimal nutrition that enhances muscle recovery is currently unclear. The type, dose, and time of intake of a nutrition supplement may all play a role in facilitating muscle recovery after eccentric exercise, but currently the best combination of these factors is unknown.

Protein is used after exercise in muscle cells for many purposes. Most significantly, it is used to build and repair tissue. Protein is not thought to be a main energy source during resistance exercise, but it has been estimated to contribute between 5-15% of energy during endurance exercise (Paul 1989). Protein can be converted to glucose through liver and muscle gluconeogenesis, with the latter only providing glucose for the local working muscle. Because physical activity improves protein utilization (Butterfield et al. 1984), protein balance is an important consideration during exercise. If muscle protein synthesis is greater than muscle protein breakdown, positive muscle protein balance prevails, and protein may be used for muscle growth and repair. However, if muscle protein breakdown is greater than muscle protein synthesis, negative muscle protein balance prevails, and sufficient protein is not available for muscle growth and repair (Evans 1991).

Exercise has a significant acute effect on protein metabolism. After eccentric exercise, both muscle protein breakdown and synthesis increase for up to 48 hours after exercise ceases (Tipton et al. 1998). For muscle protein synthesis to increase, ample amino acids must be delivered and transported into the myocytes. Increasing dietary amino acids after exercise has been reported to increase amino acid availability, and thus increased muscle protein synthesis and breakdown (Tipton et al. 1998). Indeed, the presence of increased dietary amino acids resulted in greater muscle anabolism (Tipton et al. 2001b). In addition, several studies have found that the ingestion of a protein and/or carbohydrate supplement immediately after eccentric exercise caused an acute increase in protein net balance (Gibala 2000), whereas a negative protein balance occurred in the fasted state (Wolfe 2006). Thus, exercise causes an increased requirement for dietary protein. Interestingly, a recent study showed that the rate of human muscle protein synthesis is regulated by sensing the concentration of extracellular, not intramuscular, amino acid availability (Bohe et al. 2003). However, the amount of amino acids required, the optimal amino acid profile, and the resultant net gain in muscle protein synthesis are currently unknown (Wolfe 2006). Knowing that sufficient protein is necessary for muscle anabolism after exercise, and considering that nutrients repair muscle damage, it is logical to hypothesize that increased dietary protein intake after muscle-damaging exercise may speed muscle recovery.

Previous studies have shown additional benefits of protein supplementation. When a mixture of amino acids was ingested chronically, recorded beneficial effects included faster recovery time from eccentric exercise-induced muscle fatigue, increased blood oxygen-carrying capacity (Ohtani et al. 2006), decreased bacterial/viral infections, decreased muscle/joint problems, and decreased heat exhaustion (Flakoll et al. 2004).

Currently, the current United States Recommended Daily Allowance (RDA) for protein for the average American is 0.8 g/kg per day. However, many factors influence a person's protein requirement, such as exercise intensity, exercise training, exercise type, energy balance, gender, age, timing of nutrient intake, and subsequent exercise sessions (Lemon 1998). Thus, many research studies provide evidence that exercising individuals have a higher protein requirement. One study (Meredith et al. 1989) fed 12 endurance-trained men 0.6, 0.9, or 1.2 g/kg per day of high-quality protein over three separate 10-day periods,

while maintaining training and constant body weight. Whole-body nitrogen measurements showed that all subjects were in negative nitrogen balance at the intake of 0.6 g/kg per day, seven men were in negative nitrogen balance at the intake of 0.9 g/kg per day, and only one man was in negative nitrogen balance at the intake of 1.2 g/kg per day. Researchers concluded that, regardless of the age of the subject, the estimated protein requirement was 0.94 +/- 0.05 g/kg per day. Also, they reported that protein intake increased whole body protein flux and synthesis. A 40-day study (Consolazio et al. 1975) researched two groups of men, consuming either 1.4 or 2.8 g/kg per day of protein. Subjects performed fairly heavy physical activity every day and had significant nitrogen losses from sweat. The researchers found that the group consuming 1.4 g/kg per day of protein had adequate protein, and that additional protein did not enhance work performance. However, the 2.8 g/kg per day protein intake caused increased body protein stores and muscle mass. Thus, the researchers concluded that the lower protein intake, or ~100 g of protein/day, was adequate for men performing heavy exercise. For a 75 kg man, this equals 1.33 g/kg per day. Interestingly, the researchers also noted that nutrient and nitrogen losses during profuse sweating caused calculation error that may seriously invalidate the accuracy of metabolic balance studies. Thus, because of sweating, an exact calculation of nitrogen balance during heavy exercise is virtually impossible to determine, unless nitrogen losses are accounted for in sweat.

Studies like these have led the American Dietetic Association and the American College of Sports Medicine to recommend protein intake between 1.2 to 1.4 g/kg body weight per day for endurance athletes and 1.6 to 1.7 g/kg body weight per day for resistance and strength-trained athletes (ADA 2000). Currently, there is no evidence that protein intake in these ranges will cause adverse effects in healthy individuals (Lemon 1998).

Protein Supplements Before, During, and After Exercise

Several studies have concluded that ingesting protein supplements before, during, or after exercise can decrease muscle damage and/or increase muscle recovery. Recently researchers (Sugita et al. 2003) compared the effects of ingesting an amino acid mixture or placebo for 10 days after eccentric exercise. The amino acid mixture was 5.6 g per dose, and it contained 12 amino acids, with glutamine, arginine, leucine, isoleucine, and valine composing over 50% by weight. The study included young, healthy males (19-21 years) who

completed two trials (separated by two months), with subjects ingesting one supplement after each trial. Muscle peak torque and DOMS were recorded at several time points. At certain time points, the results indicated a significant smaller decline in muscle strength (isometric peak torque) in the amino acid group compared to the placebo group. Also, most subjects reported less DOMS after ingesting the amino acid mixture. The researchers concluded that the ingestion of the amino acid mixture sped the rate of extensor muscle recovery, caused larger peak torque values, and caused decreased DOMS values. Thus, amino acids may prevent muscle damage and/or promote muscle recovery.

Another crossover study (Ohtani et al. 2006) instructed 24 untrained males to ingest either an amino acid mixture or placebo before and after endurance exercise. Subjects completed two trials (separated by four weeks), and in each trial they consumed one of the supplements. The researchers found that when subjects ingested the amino acid supplement, increases in CPK activity and muscle soreness were significantly smaller than after ingesting placebo. This study indicated that amino acid supplementation with exercise caused decreased muscle damage and/or increased muscle recovery.

A 54-day study of 387 young, healthy, male marines assessed perceived muscle soreness (DOMS) using a 1-10 continuous range self-reported evaluation scale (Flakoll et al. 2004). The marines ingested supplements (protein, carbohydrate, lipid, respectively, in grams) with predominantly protein (10, 8, 3), carbohydrate (0, 8, 3), or placebo (0, 0, 0) immediately post-exercise every other day for the entire study. On both days 34 and 54, the results indicated that the protein supplement caused significantly decreased muscle soreness compared to carbohydrate or placebo. These studies, as well as many others, indicate that protein supplements may have a significant effect on muscle recovery after exercise-induced muscle damage.

Whey Protein

Protein is available from many food sources, including both animal and plant sources. However, different sources yield protein that varies greatly in amino acid content, bioavailability, and biological value. Several studies have compared various proteins and their effects under exercise conditions. A recent study (Tipton et al. 2004) compared the effect of ingesting either 20 g of casein, 20 g of whey protein, or placebo one hour after leg

extension exercises. Blood samples showed that ingestion of both protein types caused similar increases in muscle protein balance and muscle protein synthesis. However, in a study (Morifuji et al. 2005) comparing the effects of casein and whey protein in exercising rats, researchers found that rats consuming whey protein significantly increased both their skeletal muscle glycogen content and liver glycogen content more than rats consuming casein. Interestingly, a study comparing lean body mass gains of weightlifters found that soy protein and whey protein both promoted training-induced lean body mass gain (Brown 2004). However, a review comparing several recent studies concluded that when balanced quantities of total protein and energy are consumed, milk proteins (whey and casein) are more effective than soy protein in stimulating amino acid uptake and protein turnover in skeletal muscle after resistance exercise (Phillips et al. 2005).

Effects of Specific Amino Acids and Metabolites

It has been suggested that only essential amino acids (EAA) are required for the increase in net muscle protein balance, and nonessential amino acids (NEAA) are not necessary. One study (Borsheim et al. 2002) compared the effects on net muscle protein balance from one of two doses of amino acids: either 6 g of orally administered EAA, or a mixture of 3 g EAA and 3 g NEAA. The data indicated that the pure EAA dose stimulated twice the net muscle protein balance response compared with the EAA+NEAA mixture, and the researchers concluded that NEAA are not necessary for stimulation of net muscle protein balance. Another study (Tipton et al. 1999) provided subjects with either 40 g of a mixed amino acid (EAA+NEAA) solution, 40 g EAA solution, or a placebo solution after a bout of resistance exercise. The results indicated that net muscle protein balance significantly increased with the EAA+NEAA or EAA solutions as compared with the placebo solution. However, net balance was similar for NEAA+EAA and EAA. Thus, researchers found that the NEAA mixture did not affect muscle protein balance; EAA was the determinant. It is difficult to compare the results of these two studies because the investigators used such disparate amounts of amino acids.

The branched chain amino acids (BCAA) leucine, valine, and isoleucine have unique structures. They are readily oxidized in skeletal muscle, and they are regulated in skeletal muscle by a specific complex -- the branched-chain alpha-keto acid dehydrogenase

(BCKDH) complex. Exercise activates the BCKDH complex, causing increased BCAA catabolism and possibly an increased BCAA requirement (Shimomura et al. 2006). Because of their prominent role in muscle metabolism, BCAA have often been researched as possible exercise performance enhancers. Researchers have developed a number of theories to explain how BCAA increase exercise performance. One is that BCAA delay the onset of central nervous system fatigue; another is that BCAA extend performance by serving as substrates for energy expenditure (ADA 2000). It also has been suggested that exercise increases the catabolism of BCAA, causing an increased requirement for BCAA. Supplementation with BCAA has been reported to decrease the breakdown of muscle protein during exercise, and thus a BCAA supplement may attenuate muscle damage due to exercise and increase recovery from damage (Shimomura et al. 2006).

Researchers have studied the effect of BCAA on muscle recovery after exercise. One study (Coombes et al. 2000) separated 16 male subjects into two groups: one group received BCAA supplements for 14 days before exercise, and the other group did not. Before exercise, normal blood values were recorded for both groups. After prolonged cycling exercise, however, the BCAA group had significantly decreased CPK activity, suggesting that the BCAA supplement reduced muscle damage. A recent study (Shimomura et al. 2006) demonstrated that a BCAA supplement before squat exercise decreased DOMS, suggesting that BCAA may increase muscle recovery after exercise. However, results of studies that have used BCAA are inconsistent and currently their use as exercise performance enhancers is unclear (ADA 2000).

Beta-hydroxy-beta-methylbutyrate (HMB) and alpha-ketoisocaproic acid (KIC) are metabolites of leucine. Some researchers have suggested that they also may decrease muscle damage following eccentric exercise. A recent study (van Someren et al. 2005) compared the effects of a HMB (3 g) + KIC (0.3 g) supplement vs. placebo when given daily for 14 days prior to a bout of muscle-damaging eccentric exercise. Muscle damage indices, including CPK and DOMS, were measured pre-exercise and at 1, 24, 48, and 72 hours post-exercise. Compared with placebo, the results indicated that the HMB+KIC supplement significantly decreased CPK activity and DOMS. Thus, the researchers concluded that the HMB+KIC supplement diminished the muscle damage from a single bout of eccentric exercise.

Similarly, another study (Nissen et al. 1996) compared the effects of various amounts of HMB supplements during resistance training. After measuring indicators including CPK and body composition, researchers reported that HMB in association with resistance training decreased exercise-induced muscle damage and caused increased gains in muscle function.

CARBOHYDRATE METABOLISM DURING AND AFTER EXERCISE

Muscle cells need a constant supply of energy to properly function during exercise. This energy originates most directly from carbohydrate sources. The body uses muscle glycogen as the first main source of energy, and it then shifts to blood glucose if necessary (ADA 2000). It is well known that skeletal muscle glycogen is a substrate for energy metabolism within the working muscle during exercise, but muscle-damaging exercise may result in impaired glycogen synthesis. Researchers have found (Sherman et al. 1983; Zehnder et al. 2004) an association between delayed muscle damage and impaired glycogen repletion following endurance exercise. Because muscle-damaging exercise is thought to alter muscle cell membrane integrity, eccentric exercise results in decreased insulin sensitivity and glucose availability to the muscle cell (Evans 1991). Because of these many functions of carbohydrate, researchers have investigated whether carbohydrate supplements may decrease muscle damage after exercise, but thus far evidence for this is sparse (Close et al. 2005). Currently, the carbohydrate recommendations for athletes range from 6 to 10 g/kg body weight per day (ADA 2000).

Carbohydrate Supplements Before, During, and After Exercise

Some research indicates that carbohydrate ingestion replenishes muscle glycogen to a greater extent than placebo (Tarnopolsky et al. 1997), but other research has not shown this effect. In a recent study (Zehnder et al. 2004), researchers examined the effect of post-exercise carbohydrate ingestion on glycogen repletion in 20 subjects with depleted glycogen stores. After glycogen depletion, one group of subjects performed eccentric exercise and one group rested. During the next 24 hours, both groups consumed a high carbohydrate diet. The results indicated that the high carbohydrate diet did not replenish glycogen stores after eccentric exercise.

Carbohydrate is the primary source of energy for working muscles, and thus scientists have suggested that increased carbohydrate intake before or after eccentric exercise may

decrease muscle damage and DOMS. However, some current studies have not found this evidence in controlled clinical trials. A recent study (Close et al. 2005) included 12 subjects who performed two identical downhill runs, one after a high carbohydrate diet and one after a low carbohydrate diet. After this eccentric exercise, all subjects developed similar DOMS and CPK activity regardless of treatment. The researchers concluded that carbohydrate status before exercise had no effect on muscle damage from eccentric exercise. Similarly, another study (Nelson et al. 2004) compared the effect of inadequate carbohydrate intake on muscle soreness after eccentric exercise. This study split 33 young male subjects into three groups: one glycogen depleted, one glycogen depleted and then repleted, and one received no treatment. Researchers measured DOMS and isometric force production before and after treatment. The results indicated DOMS and decreased isometric peak force for all groups, but no differences were seen among any of the groups. The researchers concluded that inadequate carbohydrate intake did not affect DOMS and muscle strength.

Unlike dietary protein, dietary carbohydrate causes a negligible increase in protein synthesis. In one study (Levenhagen et al. 2002), an intake of 15 g of carbohydrate (verses fasting) immediately post-exercise did not improve whole body or muscle protein synthesis. A set of studies compared three groups of subjects, all performing prolonged resistance leg exercises. After exercise, each group consumed either: 100 g of a protein/carbohydrate mixture, 100 g of carbohydrate, or placebo. Muscle protein balance was measured by blood samples and muscle biopsies. The researchers found that the ingestion of carbohydrate improved net leg protein balance after exercise (Borsheim et al. 2004b), but its effect was minor compared to the identical study with amino acid ingestion (Borsheim et al. 2004a). Thus, although many studies have found that carbohydrate consumed after exercise is beneficial to subjects, its usefulness compared with protein still requires additional research.

COMBINED CARBOHYDRATE AND PROTEIN SUPPLEMENTS

Protein and carbohydrate each cause specific effects on muscle metabolism. Several researchers have examined the effect of ingesting a combination protein and carbohydrate supplement, investigating the theory that an interactive effect may cause greater gains than with either singular supplement (Wolfe 2002). Consuming protein with carbohydrate does not seem to increase glycogen repletion, but it may be useful for muscle protein repair (ADA

2000). Compared to placebo, research indicated that a carbohydrate/protein supplement after exercise consistently resulted in lower CPK values (Seifert et al. 2005), with several studies providing evidence.

Researchers (Borsheim et al. 2004a) compared the effects of two isocaloric supplements: the first contained 100 g carbohydrate, whereas the second contained 77.4 g carbohydrate, 17.5 g whey protein, and 4.9 g amino acids. As assessed by blood samples and muscle biopsies, this study found that the protein + carbohydrate combination supplement stimulated muscle protein synthesis more than did the carbohydrate alone. Another study (Wojcik et al. 2001) compared the muscle damage of subjects who consumed a protein/carbohydrate, carbohydrate, or placebo beverage after eccentric exercise. The results indicated that CPK activity was least in the subjects consuming the protein/carbohydrate beverage.

A recent study (Millard-Stafford et al. 2005) compared the effect of 6% carbohydrate, 10% carbohydrate, or 8% carbohydrate + 2% protein on muscle recovery after exercise. The results showed that although CPK activity was similar among all groups, perceived muscle soreness was lower in the carbohydrate/protein group than with carbohydrate alone. Another study (Saunders et al. 2004) compared the effects of a carbohydrate beverage verses a protein/carbohydrate beverage on CPK activity in 15 male cyclists. The beverages were not isocaloric, but they were matched for carbohydrate content. Each subject, serving as his own control, completed two exercise sessions (separated by two weeks), consuming a different supplement each session. The researchers found that the peak post-exercise plasma CPK activity was 83% lower after the protein/carbohydrate trial than after the carbohydrate trial. Thus, the researchers concluded that the protein/carbohydrate beverage decreased muscle damage. However, further research is necessary to determine whether the results were because of the increased energy content of the protein/carbohydrate beverage, or if an isocaloric beverage containing protein and carbohydrate would produce the same results. From these studies, it seems fairly clear that a protein/carbohydrate supplement after exercise decreases muscle damage to a greater extent than a carbohydrate supplement.

OTHER MUSCLE RECOVERY SUPPLEMENTS

Many other nutrients have been researched as possible effectors on markers of exercise-induced muscle damage following eccentric exercise. A recent study (Rawsom et al. 2001) compared the effects of oral creatine verses placebo on indicators of muscle damage in 23 males (18-36 years) for 5 days before performing high-intensity eccentric exercise. However, the data indicated that the creatine supplement did not decrease muscle damage or enhance recovery. A similar study (Jakobi 2000) also found that short-term creatine supplement did not influence muscle force, delay time to muscle fatigue, or improve muscle recovery. Ibuprofen is an effective treatment in decreasing muscle soreness after eccentric exercise, but it did not assist in restoring muscle function (Tokmakidis 2003). Naproxen also decreased DOMS and increased peak torque, but it did not decrease CPK activity (Lecomte 1998).

SUPPLEMENT PROTOCOL SELECTION

Timing of Supplement

The timing of supplement ingestion is a very important consideration. Should the supplement be consumed days before, hours before, immediately before, during, immediately after, or hours after exercise? A recent study (Tipton et al. 2001a) examined whether an amino acid/carbohydrate supplement would cause a greater anabolic response before or after resistance exercise. Six healthy subjects each participated in two trials, either consuming the supplement immediately before or immediately after exercise. Blood samples and muscle biopsies in both trials indicated that amino acid delivery to the leg was elevated during and after exercise for two hours. However, when the supplement was ingested before exercise, amino acid delivery to the leg was significantly greater during and for the first hour following exercise. These results suggest that muscle protein synthesis is greater when the supplement is consumed immediately before exercise. In contrast, a more recent study (Tipton et al. 2006) examined whether these results also apply to the ingestion of whole protein, but the researchers found that the data were not similar. Seventeen subjects were assigned randomly to one of two groups: one consumed whey protein immediately before exercise and the other consumed whey protein immediately after exercise. The exercise consisted of 10 sets of 8 repetitions of leg extension exercise. The results indicated that

amino acid uptake was not significantly different between the groups. Thus, the response of net muscle protein balance is not affected by the timing of ingestion, and whole protein does not produce the same result as does a combination of EAA+carbohydrate supplement.

Another study (Levenhagen et al. 2001) compared the timing of ingestion after exercise. Ten subjects performed two trials, ingesting the same supplement composed of 10 g protein, 8 g carbohydrate, and 3 g fat during both trials. Subjects consumed the supplement either immediately after or three hours after one hour of exercise. Results showed that leg glucose uptake and leg protein synthesis both increased 300% more when the supplement was consumed immediately verses three hours later, indicating a significant advantage in consuming this supplement immediately after exercise. In summary, consuming nutrition supplements immediately after exercise clearly causes greater effects than consumption several hours post-exercise (ADA 2000). Further, supplement consumption immediately before or after exercise does not seem to make a significant difference.

Dose Amount

The amount of the dose administered in research studies varies considerably. Some studies administer doses that are identical for all subjects, such as 100 g carbohydrate for all subjects (Borsheim et al. 2004b). However, this may not be the best method, considering the great variability in body weight and thus different amounts of muscle mass and variation in metabolic rate from person to person. A more accurate method to dose subjects is to administer the supplement on a "gram supplement per kilogram body mass" basis. For instance, a person would receive 1.2 g supplement per kg body mass. The dose administered in studies (Williams et al. 2003; Wojcik et al. 2001; Berardi et al. 2006) varies greatly, but commonly is about 1.0 g protein/kg body weight.

A recent study compared the effects of ingesting various doses of a protein supplement on exercise-induced muscle damage (Nosaka et al. 2006). Before and after endurance exercise on the elbow flexors, subjects ingested a total of either two or ten doses of supplement. The subjects consuming only two protein doses reported no significant differences in CPK activity or muscle soreness verses consuming placebo, but the subjects consuming ten doses showed significantly lower CPK activity and muscle soreness verses

consuming placebo. Clearly, the increased number of protein supplement doses had a significant effect on muscle recovery after exercise.

Other Protocol Decisions

To perform optimally during exercise, it is vital that individuals maintain normal fluid status. Thus, before, during, and after an exercise protocol, individuals must drink adequate fluids. However, exercise of moderate duration (less than 3 hours) does not require replacement of electrolytes (ADA 2000). Current research recommends intake of carbohydrate with fluids to increase exercise performance. This is especially important for individuals who exercise after an overnight fast. It is best that the carbohydrate consumed is glucose or sucrose, because fructose alone can cause diarrhea (ADA 2000).

FURTHER RESEARCH

Although a plethora of research has been conducted on nutrient intake, metabolism, and recovery from exercise, clear conclusions have not yet been realized. Many research studies have reported convincing evidence as to the usefulness of protein and/or carbohydrate supplements after exercise, but many scientists remain unconvinced that these supplements are truly necessary or helpful (Williams 1999; Nemet et al. 2005; Kreider et a. 1993). Further research needs to be conducted to clarify the usefulness of protein and/or carbohydrate supplements after eccentric exercise to decrease muscle damage and/or increase muscle recovery.

CHAPTER III: IMPACT OF PROTEIN SUPPLEMENTATION ON MUSCLE RECOVERY AFTER EXERCISE-INDUCED MUSCLE SORENESS

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ABSTRACT

We investigated whether protein (PRO [0.4g/kg]) vs. carbohydrate (CHO [0.4g/kg]) vs. placebo nutrition supplements would alleviate muscle soreness when consumed immediately after eccentric exercise in 21 untrained men aged 18-30 years. During this double-blind randomized block study design, each subject completed three, 3-day trials (separated by $\geq$2 weeks), identical except for treatment, with each serving as his own control. Trials began with a bout of right leg eccentric exercise (Biodex), followed directly by treatment. At 0 (baseline), 24, and 48 hours, data were collected: creatine phosphokinase (CPK) from pre-exercise blood samples, subjective muscle soreness questions, and strength tests (power, torque, work.). ANOVA indicated that exercise caused mild muscle damage, evidenced by an overall day effect ($p \leq 0.0001$) for muscle soreness, with the lowest median values (0 to 10 scale) on day 1 (0.7), increasing ($p \leq 0.0001$) on day 2 (3.2), and remaining elevated on day 3 (3.4). We also noted an overall day effect ($p \leq 0.0001$) for CPK, with lowest median values (U/L) on day 1 (136), increasing ($p \leq 0.0001$) on day 2 (235), and remaining elevated on day 3 (189). ANOVA revealed no significant treatment effect on indicators of soreness or damage during recovery. Our results indicated that a PRO or CHO supplement after exercise causing

mild muscle damage did not facilitate muscle recovery in adequately nourished, healthy young men.

KEY WORDS: eccentric exercise, creatine phosphokinase, muscle damage

INTRODUCTION

In general, unaccustomed exercise causes muscle soreness, which usually begins within 24 hours and peaks within 48 hours after exercise. Eccentric exercise, which occurs when a skeletal muscle lengthens as it produces force, particularly causes muscle damage. Common responses to exercise-induced muscle damage include increased creatine phosphokinase (CPK) (Gleeson et al. 1995), increased muscle soreness (Newham et al. 1983b), decreased peak torque, decreased power output, and decreased range of motion (Chapman et al. 2006; Vincent et al. 1997; Paschalis et al. 2005b). Effective eccentric exercises include running downhill (Newham et al. 1986), stepping down (Newham et al. 1983a), or action of the elbow flexors (Chapman et al. 2006) or knee extensors (Paschalis et al. 2005a) on a dynamometer. The extent of muscle damage, however, depends upon the gender (Pincivero et al. 2003), training status (Vincent et al. 1997), and/or nutrition status of the individual.

During exercise, amino acids are used to build and repair tissue as well as to supply energy, whereas carbohydrate is the primary energy source for muscle contraction. In the event that amino acids and/or carbohydrate are limiting during the course of exercise, optimal muscle protein synthesis and turnover (Ohtani et al. 2006; Tipton et al. 1998) may not proceed and muscle recovery may be impeded. Hence, nutrition supplements before or after eccentric exercise may significantly impact muscle damage and muscle recovery, and specifically a protein supplement has considerable potential to decrease muscle damage and soreness (Sugita et al. 2003; Ohtani et al. 2006). In previous studies (Tipton et al. 1998; Tipton et al. 2001b; Gibala 2000), increases in whole body and muscle protein accretion were noted when a protein supplement was ingested immediately following exercise. Studies reported that amino acid supplements given after exercise led to more rapid recovery from muscle fatigue, decreased plasma CPK activity, decreased muscle soreness, and lessened

exercise-induced proteolysis (Ohtani et al. 2006; Nissen et al. 1996; Flakoll et al. 2004) Further research (Levenhagen et al. 2001) suggested that post-exercise protein ingestion was most beneficial to muscle protein accretion when consumed immediately after exercise versus several hours later. Researchers (Sherman et al. 1983; Zehnder et al. 2004) also have investigated whether carbohydrate supplements decrease muscle soreness because of increased energy availability or muscle glycogen repletion. However, most researchers suggested that inadequate carbohydrate intake did not affect CPK activity (Close et al. 2005), muscle soreness (Nelson et al. 2004; Close et al. 2005), and muscle strength (Nelson et al. 2004).

The purpose of this study was to determine whether distinct nutritional supplements (protein vs. carbohydrate vs. placebo) cause different effects on muscle recovery after exercise-induced muscle soreness in untrained healthy men aged 18 – 30 years. Our hypotheses were that a protein supplement would promote more rapid and greater muscle recovery after a single bout of exercise-induced muscle soreness compared with carbohydrate or placebo, and that a carbohydrate supplement would promote more rapid and greater muscle recovery after a single bout of exercise-induced muscle soreness compared with placebo.

METHODS

Subjects

Volunteers were required to be non-smokers, untrained, 18 –30 year old males, with a BMI in the range of 18.0 – 29.9 kg/m^2. Subjects were required to refrain from medication or vitamin/mineral supplement use during the study. Each subject served as his own control. We excluded subjects who had recently increased their level of resistance training prior to participating in this study, whereas those who had maintained a relatively constant degree of physical activity were allowed to participate.

Pre-baseline

Screening. The Human Subjects Institutional Review Board at Iowa State University approved the study (IRB ID#05-341). The study design, purpose, and potential risks were explained to each subject, with an opportunity for each to ask questions. Prior to screening,

each subject signed an Informed Consent document. All portions of the study were carried out in the Human Metabolic Unit (HMU) on the Iowa State University campus. Prior to enrollment in the study, each volunteer was screened to determine eligibility and measurements were recorded for height, weight, blood pressure, and heart rate. Using height and weight, BMI was calculated for each subject. Bio-electrical impedance analysis (BIA) was used to assess body composition, with three measurements recorded and averaged for each subject. To further determine eligibility and screen for past or current health problems, each potential subject completed a medical history questionnaire and a physical activity questionnaire. To confirm eligibility, each potential subject also had an initial blood draw by venipuncture (8.5 mL vacutainer) that was analyzed by a certified clinical laboratory (Laboratory Corporation of America; Kansas City, MO) with chemistry profile results recorded for each subject. We excluded volunteers with evidence of hypertension, liver disease, kidney disease, diabetes, asthma, and/or abnormal blood values. We enrolled volunteers in the study who met all inclusion and exclusion criteria.

During screening each volunteer participated in an exercise test to measure leg flexion/extension muscle strength on the dynamometer (Biodex System 3 Isokinetic Dynamometer, Biodex Medical Systems; Shirley, NY), as well as participated in a short training session that explained the future exercise protocol. Muscle strength indicators recorded during this baseline test and all subsequent exercise tests were: extension peak torque, extension maximum repetition total work, extension average power, extension average peak torque, flexion peak torque, flexion maximum repetition total work, flexion average power, and flexion average peak torque.

Instructions for subjects. Subjects were instructed to eat their normal diets before and during the study, but they were only allowed to drink two alcoholic drinks each day, beginning three days before and lasting until the completion of the trial. Also, subjects were instructed to refrain from any new or unusual physical activity beginning one week before and lasting until the completion of the trial. Each participant completed three, 3-day trial periods. Beginning three days before his first visit to the HMU, each subject began keeping a written 24-hour diet record. This record continued until the first day of the trial (time = 0 hours), and thus the record did not include the protein or carbohydrate supplement administered during

the trial. Each subject also began recording his 24-hour physical activity record the day prior to the first day of the trial. This record continued until the end of each trial.

Study Design and Protocol

Supplement intervention. This double-blind, crossover study was designed to compare the effects of protein, carbohydrate, and placebo beverage supplements on markers of muscle damage (CPK, muscle soreness questionnaire, muscle strength test). Each subject served as his own control, and thus completed the identical protocol three times, consuming one of the three supplements each time. The supplements were administered in random order, thus preventing a trial sequence effect. The supplements were composed of either: 1) Protein: whey protein (0.4 g/kg body weight) with cherry flavoring (Nectar! Whey Protein Isolate, Syntrax, SIO3 Inc.; Cape Girardeau, MO), dissolved in 1 cup water; 2) carbohydrate: sugar (0.4 g/kg body weight and cherry Kool-Aid® (Kraft Foods Global, Inc.; Glenview, IL), dissolved in 1 cup water; 3) placebo: Splenda® (McNeil Nutritionals, LLC; Ft. Washington, PA) (0.0485 g/kg body weight) and cherry Kool-Aid®, dissolved in 1 cup water. Protein and carbohydrate treatments were isocaloric.

Exercise protocol. Each trial began with the fasted subjects arriving at the HMU in the early morning (0 hours). Researchers recorded measurements of height, weight, blood pressure, and heart rate, and a phlebotomist drew an 8.5 mL blood sample by venipuncture (vacutainer). Each subject recorded his initial muscle soreness, performed a short muscle strength test on the dynamometer identical to the screening test, and completed a level of exertion evaluation. Perceived muscle soreness was assessed using a continuous-range evaluation. The subjects self-reported their soreness on a scale from 0-10 (0 representing “not at all sore” and 10 representing “extremely sore”). Perceived level of exertion was assessed using a continuous-range evaluation. The subjects self-reported their soreness on a scale from 0-10 cm (0 representing “no exertion” and 10 representing “maximum exertion”). The purpose of this evaluation was to encourage subjects to exert maximal exertion and to compare exertion levels between trials.

The subject performed the muscle-damaging eccentric exercise protocol, consisting of extending and contracting the right leg against a controlled force provided by the dynamometer. The dynamometer was set in passive mode and thus did not move without

force placed against it. Subjects were encouraged to perform maximal voluntary effort during all sets, performing 10 sets of 10 repetitions of leg extensions at 60 deg/s at 120% of maximum peak torque recorded during the screening muscle strength test. A one-minute break occurred between all intervals, and the entire protocol lasted about 15 minutes. After the first and last exercise intervals, the subject completed an evaluation of his level of exertion. Immediately following the exercise protocol, the subject drank a beverage, either protein, carbohydrate, or placebo. The subject then completed another evaluation of his muscle soreness and provided another 8.5 mL blood sample. Thirty minutes after exercising, each subject had his blood pressure and heart rate taken. The subject was then allowed to leave the unit but was asked to refrain from eating any food until at least 45 minutes after the completion of exercise. Water was provided ad libitum throughout the protocol.

Subjects returned to the HMU in a fasted state the following two days (24 and 48 hours), at approximately the same assigned time as the first day. Weight, blood pressure, and heart rate were recorded, and an 8.5 mL blood sample was taken. Each subject completed another muscle strength test, an evaluation of level of exertion, and an adverse events questionnaire. Muscle soreness evaluations were completed before and after the strength test.

At least two weeks after the first trial, each subject returned and repeated the exact same protocol, except he consumed a different treatment beverage. At least two weeks later, each subject again returned, and ingested the third beverage option. During the three days before the second and third trials, each subject was asked to consume the identical diet he consumed during the first trial period.

Analysis of Records

All 24-hour diet records were analyzed using Nutritionist Pro™ (Axxya Systems; Stafford, TX). All 24-hour physical activity records were analyzed by quantifying subjects' daily activities (METs) as rest, light activity, moderate activity, or heavy activity. These category distinctions were based on standard lists of various activities (Ainsworth et al. 1993).

Analysis of Blood Samples

Phlebotomists collected blood samples from fasted (10 hours) participants between 6:00 and 8:30 a.m. We separated plasma from whole blood by centrifuging for 10 minutes

(room temperature) at 1500 x g and stored aliquots at -80^{o}C until analyses. Initial blood samples were analyzed by a certified clinical laboratory (LabCorp; Kansas City, Kansas) for a complete blood count (CBC) with differential and general chemistry panel (ChemScreen). We determined plasma CPK activity in duplicate using a Creatine Kinase Reagent Set (Pointe Scientific, Inc.; Canton, MI), according to the manufacturer's instruction. The absorbance of the samples was determined on a Beckman spectrophotometer (Beckman Coulter, Inc.; Fullerton, CA) and compared to standards provided by the company. The spectrophotometer measured the rate of NADH formation (measured at 340 nm), directly proportional to serum CPK activity, as illustrated below:

$$\text{ADP + Creatine Phosphate} \xrightarrow{\text{CPK}} \text{Creatine + ATP}$$

$$\text{ATP + Glucose} \xrightarrow{\text{Hexose Kinase}} \text{ADP + Glucose-6-Phosphate}$$

$$\text{Glucose-6-Phosphate + } NAD^{+} \xrightarrow{G_6PDH^{*}} \text{6-Phosphogluconate + NADH + } H^{+}$$

*****glucose-6-phosphate dehydrogenase

Statistical Analysis

Statistical analyses were performed using SAS (version 9.1; Cary, NC), with results considered statistically significant at $p \leq 0.05$. Descriptive statistics included means for normally distributed data (height, weight, level of exertion, dietary values, physical activity values, and muscle strength values) and medians for data that were not normally distributed (CPK and muscle soreness). To determine whether day, trial, or treatment exerted an effect on CPK or muscle soreness, analysis of variance (ANOVA) was used. We used a mixed model, with fixed effects for day, trial, and treatment, with incorporation of a random effect for subject.

RESULTS

Initial subject characteristics are reported in Table 1. All subjects met the inclusion and exclusion criteria of the study. Subjects included 21 healthy, young (20 – 28 years) males

with BMI ranging from 19.8 to 29.9. An important goal of the study was to maintain similar circumstances throughout all trials to minimize potential confounding from various extraneous factors. Thus, each subject was asked to maintain a similar dietary intake and physical activity level prior to each trial, as well as identical eccentric exertion during all three trials. At baseline, the ANOVA revealed no statistical difference among the treatments (protein, carbohydrate, placebo) for subject characteristics/activities (Table 2).

ANOVA was performed with data categorized by day (0, 24, or 48 hours) of the trial, trial number, and treatment type. The main indices of muscle damage after the eccentric exercise protocol were CPK (U/L) and subjective muscle soreness evaluations (0-10 cm scale), although we also assessed exercise measures of strength from the Biodex. It was clear that the eccentric exercise protocol caused muscle damage (Figures 1 and 2). The ANOVA for overall day effect revealed significant differences for CPK ($p \leq 0.0001$) and muscle soreness ($p \leq 0.0001$), as well as 6 (p ranged from 0.007 to 0.034) of 8 muscle strength tests, with smaller values for muscle soreness on day 1 than day 2 or 3. Clearly, the eccentric exercise effectively achieved muscle damage. In examining the overall trial effect, we noted several significant differences among trials. The ANOVA for overall trial effect showed significant differences for muscle soreness ($p \leq 0.0001$) and for 6 (p ranged from ≤ 0.0001 to 0.017) of 8 exercise muscle strength tests. Specifically, greater muscle damage occurred during trial 1 than during trial 2 or 3, as evidenced by statistically significant differences in muscle soreness values between trials 1 and 2 ($p \leq 0.0001$) and between trials 2 and 3 ($p=0.0002$), as well as greater CPK values ($p=0.039$) and several muscle strength indices in trial 2 compared with 1. We analyzed CPK activity and muscle soreness evaluation by treatment type, as depicted graphically (Figures 1 and 2). However, examining the overall treatment effect, we documented no significant differences as indicated by either CPK or muscle soreness values. Thus, the eccentric exercise protocol produced significant muscle damage with a documented trial effect, but treatment type had no effect on muscle soreness or recovery.

DISCUSSION

Contrary to other studies (Sugita et al. 2003; Ohtani et al. 2006), the results of this study indicated no significant effect of treatment on muscle damage, soreness, or recovery after moderate eccentric exercise (Figures 1 and 2). However, similar to previous studies (Gleeson et al. 1995; Newham et al. 1983b; Chapman et al. 2006; Vincent et al. 1997; Paschalis et al. 2005b), the eccentric exercise protocol successfully induced muscle damage, as evidenced by increased CPK activity and perceived muscle soreness values between day 1 and days 2 and 3 of sequential trials. Also, not surprisingly as previously explained (Byrnes et al. 1985), we documented a trial effect, with significantly greater muscle damage during and after trial 1 compared with trials 2 or 3.

Although several studies (Sugita et al. 2003; Ohtani et al. 2006; Flakoll et al. 2004; Wojcik et al. 2001; Millard-Stafford et al. 2005) indicated that protein and/or carbohydrate supplements after eccentric exercise decreased muscle damage compared with placebo, some authors (Kreider et al. 1993; Williams 1999) remain skeptical that protein and/or carbohydrate supplements cause increased muscle recovery. Several possible factors may explain why our data did not indicate significantly decreased muscle damage after protein and/or carbohydrate supplement intake.

Perhaps the main reason we did not document a treatment effect was because our exercise protocol induced moderate, not severe, muscle damage. Our objective was to create muscle damage similar to that of "real life"; it was not our goal to design an exercise protocol that would induce excruciating pain caused by muscle damage. Our aim was to determine whether a protein supplement would increase muscle recovery subsequent to a realistic exercise protocol. Thus, we chose to cause mild muscle damage using right leg eccentric exercise on a dynamometer, with a protocol similar to recently published studies (Paschalis 2005a; Babul et al. 2003). Other studies using alternative eccentric exercise protocols created more severe muscle damage, indicated by CPK activity as high as 1500 U/L from downhill walking (Newham et al. 1986) or as high as 34,500 IU/L from eccentric stepping (Newham et al. 1983a). Although this severe damage may have produced an effect in response to treatment, our aim was not to determine the response to protein by inducing severe pain or muscle damage.

Another possible explanation for the lack of treatment effect may be the small dose size provided to the well-nourished subjects in this study. Because our objective was to conduct a realistic study applicable in practical situations, we chose to administer 0.4 g protein/kg body mass. It is unlikely that our participants' diets were lacking in either energy or protein, as evidenced by their reported energy (~7934 kJ/d) and protein (78 – 83 g/d) intake from their three day, 24-hour diet records. If anything, these values are an underestimate of energy intake, given that study participants typically underreport dietary intake (Jonnalagadda et al. 2000). One would expect that in well-nourished individuals, the extracellular pool of amino acids would not be particularly limiting, except perhaps after a long-term fast (Bohe et al. 2003). Hence, a relatively small amount of protein supplements may not exert a sufficient effect on extracellular amino acid concentrations and thus influence intracellular protein synthesis subsequent to moderate muscle damage. The dose administered in research studies (Williams et al. 2003; Wojcik et al. 2001; Berardi et al. 2006) varies greatly, but it is commonly ~1.0 g protein/kg body weight. However, studies often administer much larger doses. A recent study (Nosaka et al. 2006) compared the effect of ingesting various doses of a protein supplement on exercise-induced muscle damage. Before and after endurance exercise using the elbow flexors, subjects ingested a total of either two or ten doses of protein supplement. The subjects who consumed only two protein doses versus placebo reported no significant difference in CPK activity or muscle soreness, but the subjects who consumed ten protein doses verses placebo showed significantly lower CPK activity and muscle soreness. Clearly, the greater but not lesser amount of protein in supplement form had a significant effect on muscle recovery after exercise.

This study had several limitations in that the participants were self-selected, 20 – 28 year old, well-nourished, healthy male volunteers. Hence, the results may not be applicable to the entire male population, and conclusions from the data cannot be drawn for females. Another limitation was that the subjects were free-living men, and thus we could not monitor the subjects' physical activity or dietary intake. Although subjects were instructed to refrain from physical activity for one week before each trial, their actual physical activity was not quantified. Thus, their usual physical activity may have had an undeterminable effect on muscle soreness and CPK activity. Although each subject was instructed to repeat his three

day recorded dietary intake from to the first trial during the second and third trials, this did not occur in practice in a majority of the subjects. Also, the exercise bouts were performed on a Biodex machine, which uses a person's own resistance to determine his/her level of power output. Although subjects were strongly encouraged to work "as hard as they can", the work performed from subject to subject undoubtedly varied. However, because each subject served as his own control, this should not have confounded the overall results, as long as each subject performed similarly from trial to trial. In addition, we evaluated a relatively small number (N=21) of subjects, although similar studies (Zainuddin et al. 2006; Paschalis et al. 2005b; Eston et al. 1996; Nelson et al. 2004) indicated a response to treatment, with subject numbers ranging from 5 to 20. However, a trial with a greater number of subjects would provide greater statistical power for detecting small changes, which may not have been detectable because of inherent inter-individual variability.

CONCLUSION

In conclusion, a protein or carbohydrate supplement consumed after moderate eccentric exercise did not significantly affect muscle damage or recovery. Further research is needed to determine whether significant changes may be realized with greater muscle damage, larger dose sizes, or with a different sample of subjects.

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Table 1. Subject Characteristics at Baseline

	Mean ± SD	**Range**
Age (years)	23 ± 2	20 – 28
Weight (kg)	79.2 ± 10.1	63.7 – 98.2
Height (cm)	179.6 ± 5.4	170.1 – 191.2
Body Mass Index (kg/m2)	24.6 ± 3.0	19.8 – 29.9
Fat Free Mass (kg)	63.7 ± 5.3	53.9 – 73.4
Fat Free Mass (% mass)	80.1 ± 4.0	72.0 – 88.5
Fat Mass (kg)	16.2 ± 5.0	8.4 – 27.1
Fat Mass (% mass)	19.9 ± 4.0	11.5 – 28.0

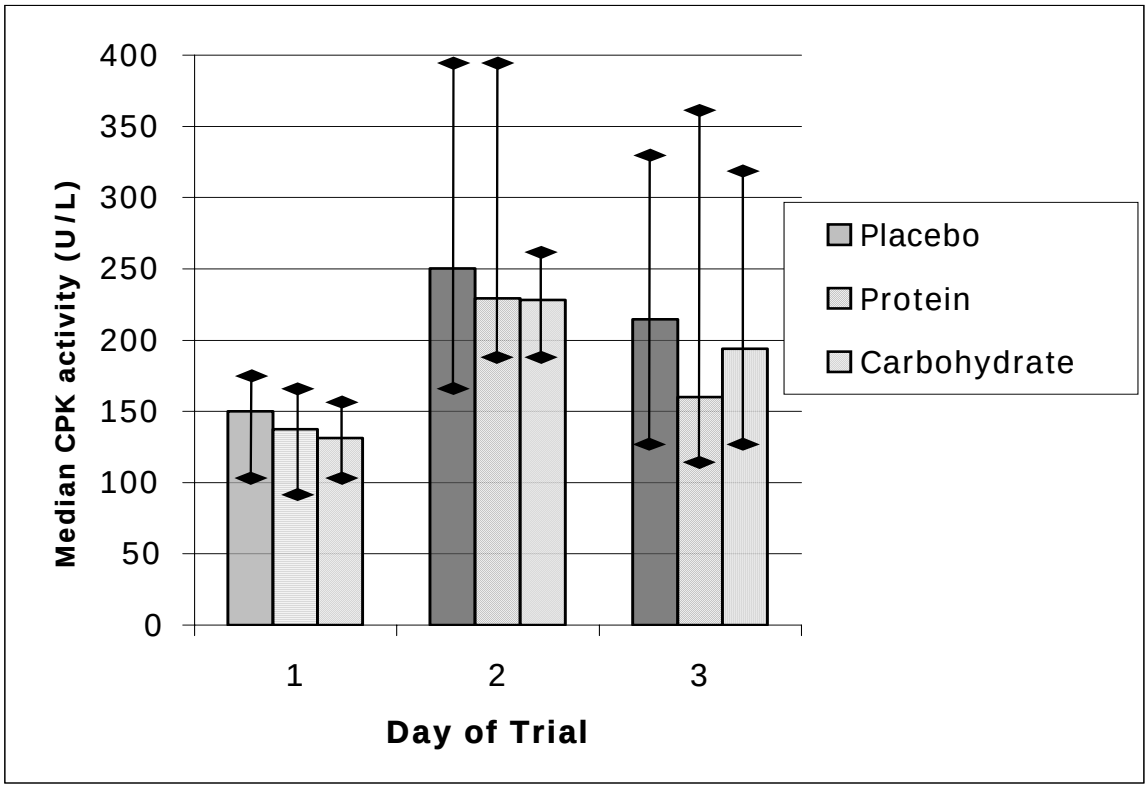
Table 2. Dietary Intake[1] of Subjects at Baseline According to Treatment

Potential Confounding Factors	**Treatment Group**			**Comparison Among Groups[2]**
	Placebo	**Protein**	**Carbohydrate**	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**p-value**
Energy (kJ/day)	7934 $\pm$ 1800	8093 $\pm$ 1775	8273 $\pm$ 2403	0.86
Protein (g/day)	78 $\pm$ 17	75 $\pm$ 17	83 $\pm$ 21	0.41
Carbohydrate (g/day)	236 $\pm$ 66	244 $\pm$ 56	248 $\pm$ 92	0.87
Fat (g/day)	73 $\pm$ 26	74 $\pm$ 36	81 $\pm$ 27	0.66

[1] Does not include protein or carbohydrate supplement intake

[2]ANOVA indicated that there were no significant differences among treatment groups.

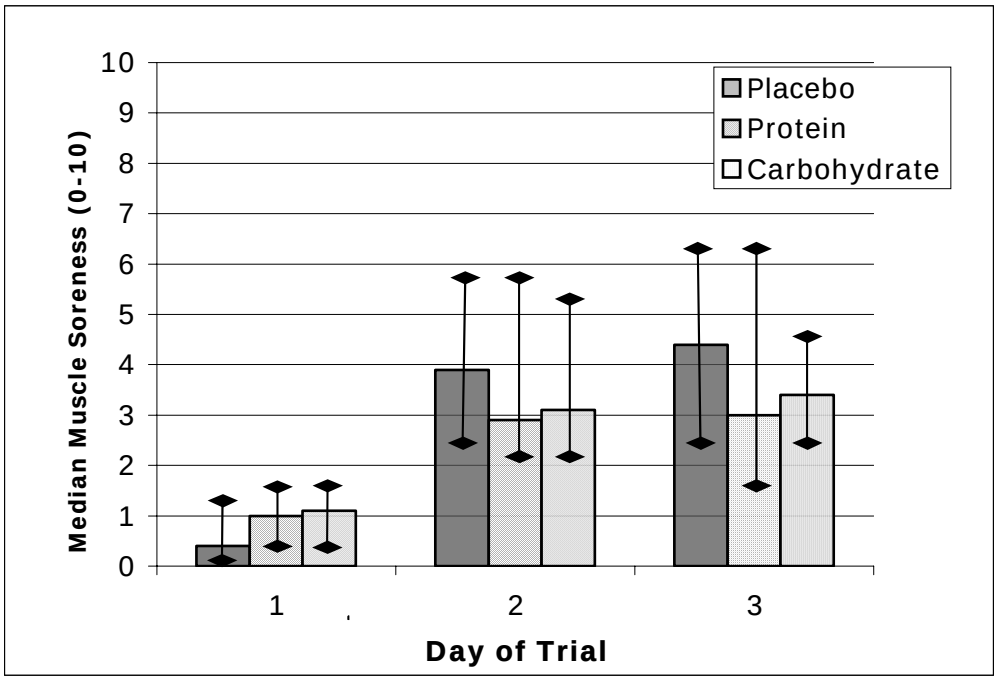
Figure 1. Serum CPK Activity (U/L) According to Treatment



Day	Treatment[1]	Min	25th quartile	50th quartile	75th quartile	Max
1	Placebo	40	101	150	173	479
1	Protein	46	95	138	170	463
1	Carbohydrate	53	112	131	158	290
2	Placebo	103	159	251	391	924
2	Protein	86	184	229	394	836
2	Carbohydrate	111	180	228	258	1172
3	Placebo	92	139	214	325	2241
3	Protein	57	120	160	359	572
3	Carbohydrate	93	131	194	314	844

[1]ANOVA indicated that there was no significant difference in CPK activity among the three treatment groups.

Figure 2. Muscle Soreness Evaluations (0-10 cm scale) According to Treatment



Day	Treatment[1]	Min	25th quartile	50th quartile	75th quartile	Max
1	Placebo	0	0.2	0.4	1.3	4.6
1	Protein	0	0.4	1	1.6	2.3
1	Carbohydrate	0	0.4	1.1	1.6	2.7
2	Placebo	1.2	2.4	3.9	5.8	8
2	Protein	0.9	2.3	2.9	5.6	7
2	Carbohydrate	0.8	2	3.1	5.4	9.3
3	Placebo	1	2.7	4.4	6.2	8.7
3	Protein	0	1.8	3	6.3	9.9
3	Carbohydrate	1.7	2.3	3.4	4.8	8.7

[1]ANOVA indicated that there was no significant difference in muscle soreness among the three treatment groups.

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CHAPTER IV: GENERAL CONCLUSIONS

In conclusion, a protein or carbohydrate supplement consumed after moderate eccentric exercise did not significantly affect muscle damage or recovery. Further research is needed to determine whether significant changes may be realized with greater muscle damage, larger dose sizes, or with a different sample of subjects.

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APPENDIX A: INFORMED CONSENT DOCUMENT

Title of Study: Impact of Protein Supplementation on Muscle Recovery after Exercise-Induced Muscle Soreness

Investigators: Elizabeth C. Dahlstrom, D. Lee Alekel, PhD, Rick Sharp, PhD, Shawn Baier, MS

This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION

The purpose of this study is to determine how nutrition affects muscle soreness due to exercise and muscle recovery. This study will demonstrate whether a difference in muscle soreness and recovery occurs when a person consumes either a protein, energy, or placebo supplement after exercise-induced muscle soreness. Increased muscle recovery is a desirable and beneficial situation, and therefore this study hopes to demonstrate that a protein or energy supplement increases muscle recovery. You are being invited to participate in this study because you are a healthy 18 – 30 year old male and able to exercise.

DESCRIPTION OF PROCEDURES

If you agree to participate in this study, the study will last for about 2 months and involve three 3-day trial periods. This study will involve 9 visits to the Human Metabolic Unit (HMU) in the Human Nutritional Sciences Building on the Iowa State University campus. You will receive a one-page outline of the study procedures attached to this form. During the study you may expect the following:

You will be screened as a potential participant prior to enrollment in the study. Screening includes the following: height, weight, vital signs (blood pressure and heart rate), and a medical history questionnaire. You will also participate in a short training session on the exercise machine that will be used in this study.

During this training session, you will sit on an exercise machine and perform a leg flexion/extension test. This will consist of extending and contracting your leg about 10 times at a pace that is comfortable to you. This training session will allow you to experience what the machine feels like and prepare you for the actual experiment.

Also at this time, your diet will be discussed. You will consume the same diet during each of the 3-day trial periods. Specific food choices will be left largely up to you, except you will be asked to limit alcoholic beverages to two drinks per day while you are recording your dietary intake. Also, you will be asked not to consume any alcohol during each of the 3-day trials.

Once these screening tests are completed and you meet the entrance criteria, you will be asked to participate in the study. Specific dates and times will be arranged according to your schedule for trial visits.

You will begin the trial by keeping a written record of your diet starting three days before you visit the HMU. This record will continue until you first visit the HMU for the first 3-day trial. You will record your physical activity beginning one day before your first visit to the HMU.

You will come to the HMU on the following day (time = 0 hours) in the morning, after an overnight fast. You will give a small blood sample (less than two tablespoons), have measurements of height, weight, vital signs, and body composition, and fill out a written muscle soreness evaluation.

Body composition will be assessed by bio-electrical impedance analysis (BIA). With BIA, two electrodes are attached to the foot and two electrodes are attached to the hand. By measuring the small electrical field created between these electrodes, body composition (fat and lean tissue) is estimated from the body's resistance to this field.

You will then be instructed to perform a leg flexion/extension test which will exercise your right leg muscles to induce muscle soreness. This test consists of extending and contracting your right leg against a controlled force for ten one-minute intervals, with a one minute break between intervals. After each exercise interval, you will fill out a written level of exertion evaluation. Immediately following the exercise, you will drink a beverage containing either protein, carbohydrate, or a placebo. You will then give another small blood sample (less than two tablespoons). You will then be asked to fill out another written muscle soreness evaluation. Thirty minutes after exercising, your vital signs will be taken again. You will be allowed to leave, and we will provide you with bottled water. However, we ask that you refrain from eating any food until at least 60 minutes after the completion of exercise.

You will return to the HMU on the following day (time = 24 hours) at approximately the same assigned time as the first day. This will be in the morning after an overnight fast. You will again give a small blood sample (less than two tablespoons) and your weight and vital signs will be measured. You will fill out a muscle soreness evaluation and an adverse events questionnaire. You will then perform a short leg flexion/extension test to evaluate your leg strength. This will require you to extend and contract your leg about 10 times to measure muscle strength. Your muscles will not be worked to induce soreness this time. Following this strength test, you will again fill out a level of exertion evaluation, a muscle soreness evaluation, and you will be allowed to leave.

You will again return to the HMU on the following day (time = 48 hours) at approximately the same assigned time in the morning after an overnight fast. You will again give a small blood sample (less than two tablespoons), and your weight and vital signs will be measured. You will fill out a muscle soreness evaluation and an adverse events questionnaire. You will again perform the same leg flexion/extension test to evaluate your leg strength. Following

this strength test, you will again fill out a level of exertion evaluation and a muscle soreness evaluation. After these tests, you will stop your physical activity record and the first trial will be finished.

Approximately 2 weeks later, you will be asked to come to the HMU again and perform a second trial, consisting of the same set of tests from 0 hours to 48 hours. During this trial however, you will be given a different beverage to drink. You will be asked to consume the same diet as in the first 3-day trial, at times identical to those in the first 3-day trial.

Approximately 2 weeks after the second trial, you will be asked to come to the HMU again and perform a third trial, consisting of the same set of tests from 0 hours to 48 hours. During this trial, you will be given a different beverage to drink**.** You will be asked to consume the same diet as in the first 3-day trial, at times identical to those in the first 3-day trial.

During all surveys and questionnaires, you may skip any question that you do not wish to answer or that makes you feel uncomfortable.

RISKS

While participating in this study you may experience the following risks:

(a) Bio-electrical impedance analysis to estimate body composition (fat and lean masses) is a painless, non-invasive test. You will be asked to lie still while a technician tapes electrodes to your hand and foot. A very small electrical current will be passed through your body. You will not be able to feel the current. You will be asked not to wear jewelry, hosiery (panty hose) or clothing with metal buttons or buckles, as they may interfere with the test. The entire procedure lasts about 5 minutes.

(b) The leg flexion and extension test will be conducted on a Biodex exercise machine by standard muscle-soreness inducing procedures, which requires the subject to exercise his leg using concentric and eccentric exercises. This may result in short-term soreness, but no long-term injury is expected. Risk of injury from exercise will be minimized by having the subject exercise at a pace that is comfortable for him with trained personnel monitoring the exercise at all times.

(c) Venipuncture, or blood collection, may be associated with hematoma, local discomfort, and on rare occasion, infection. Standard techniques will be used by a trained phlebotomist to minimize these undesirable outcomes. The total amount of blood collected for each trial will be less than 8 tablespoons, and thus will be less than 24 tablespoons for the entire study.

The risks from consuming the beverages are minimal and no greater than the risks of normal food consumption. The beverages consist of food constituents that are commercially available.

BENEFITS

If you decide to participate in this study there may be no direct benefit to you. It is hoped that the information gained in this study will benefit society by advancing the knowledge of how nutrition impacts exercise and muscle soreness. Specifically, it will help determine the role of protein and carbohydrate in muscle recovery after exercise. This information will be useful to society because it may discover a way to increase the speed of muscle recovery after exercise. Also, you will be offered the results of the body composition assessment.

COSTS AND COMPENSATION

You will not bear any costs from participating in this study. You will be compensated for participating in this study.

- You will be compensated $25.00 for each 3-day trial that you complete, with a bonus of $25.00 for completing all three trials. If all trials are completed, you will receive a total of $100.00.

PARTICIPANT RIGHTS

Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide not to participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

RESEARCH INJURY

Emergency treatment of any injuries that may occur as a direct result of participation in this research is available at the Iowa State University Thomas B. Thielen Student Health Center, and/or referred to Mary Greeley Medical Center or another physician or medical facility at the location of the research activity. Compensation for any injuries will be paid if it is determined under the Iowa Tort Claims Act, Chapter 669 Iowa Code. Claims for compensation should be submitted on approved forms to the State Appeals Board and are available from the Iowa State University Office of Risk Management and Insurance.

CONFIDENTIALITY

Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. However, federal government regulatory agencies [e.g., FDA] and the Institutional Review Board (a committee that reviews and approves human subject research studies) may inspect and/or copy your records for quality assurance and data analysis. These records may contain private information.

To ensure confidentiality to the extent permitted by law, the following measures will be taken

- Subjects will be assigned a unique subject number, which will be used on files instead of their name.
- Identifiers will be kept with the data.
- Only the principal investigator and assistants will have access to study records and identification codes, which will be kept in a locked office in a filing cabinet as well as on a password protected computer file.
- Data will be retained until approximately the end of 2008.
- If the results are published, your identity will remain confidential.

QUESTIONS OR PROBLEMS

You are encouraged to ask questions at any time during this study.

- For further information about the study contact Elizabeth Dahlstrom (515-294-3932), Dr. Rick Sharp (515-294-8650), or Dr. D. Lee Alekel at (515-294-3552).
- If you have any questions about the rights of research subjects or research-related injury, please contact Ginny Austin Eason, IRB Administrator, (515) 294-4566, austingr@iastate.edu, or Diane Ament, Research Compliance Officer (515) 294-3115, dament@iastate.edu.

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SUBJECT SIGNATURE

Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent upon request.

Subject's Name (printed) _____

_____ _____

(Subject's Signature) (Date)

INVESTIGATOR STATEMENT

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____ _____

(Signature of Person Obtaining Informed Consent) (Date)

APPENDIX B: MEDICAL HISTORY/PHYSICAL ACTIVITY QUESTIONNAIRE

Medical History Form

STUDY: Impact of Protein Supplementation on Muscle Recovery after Exercise-Induced Muscle Soreness

Subject Name:_____Date:_____

Subject ID Number: _____

Home Address: _____

Street

_____

City State Zip

Home Phone:_____

Emergency Information:

Personal Physician: _____

Contact in case of Emergency: _____

Relationship: _____ Phone: _____

Personal Information:

Age:_____ Date of Birth_____/_____/_____
Mo. Day Year

Sex: ___ male
___female

Race: ___ White
___ African American
___ Asian
___Native Hawaiian/ Pacific Islander
___American Indian/ Alaska Native

Ethnic Background: ___ Hispanic/Latino
___ Not Hispanic/Latino

Highest Level of Education:
___Elementary ___Jr. High School ___High School ___College ___Post College

Overall Health:

1. How would you rate your present health condition?
____Poor ____Fair ____Good ____Excellent

2. Typically, how many days per year are you sick enough to stay in bed? _____

3. Has your weight changed more than 10 lbs. in the last 12 months? __Yes __No

4. Have you ever smoked?
___Never ___Not now, but more than 12 months ago
___Not now, but within the past 12 months ___Yes, currently smoking

5. Have you ever used any tobacco product on a regular basis?___ yes___ no
If yes, what product did you use and for how long?
Product: _____ Years: _____

Family Health History

Date of Last Physical Exam: ____/____/____

A. If any members of your immediate family have or have had any of the following conditions, indicate their age at the time of the event:

	Father	Mother	Brother(s)	Sister(s)
Heart Attack	_____ yr	_____ yr	_____ yr	_____ yr
Stroke	_____ yr	_____ yr	_____ yr	_____ yr
Coronary Artery Disease	_____ yr	_____ yr	_____ yr	_____ yr
If deceased, age at death	_____ yr	_____ yr	_____ yr	_____ yr

B. Indicate if any members of your immediate family have or have had the following conditions by marking the appropriate lines:

	Father	Mother	Brother(s)	Sister(s)
High Blood Pressure	_____ yr	_____ yr	_____ yr	_____ yr
High Cholesterol	_____ yr	_____ yr	_____ yr	_____ yr
Diabetes	_____ yr	_____ yr	_____ yr	_____ yr
Obesity	_____ yr	_____ yr	_____ yr	_____ yr

Personal Medical History

Answer the following questions, indicating the month and year of the event or diagnosis where appropriate.

	Yes	No	Mo/Yr
1. Has a doctor ever told you that you have heart disease?	___	___	___/___
2. Have you ever had a heart attack?	___	___	___/___
3. Have you ever had chest pain?	___	___	___/___
4. Do you have a cardiac pacemaker?	___	___	___/___

5. Has your doctor ever told you that you have
   a heart valve problem? ___ ___ ___/___
6. Have you had a heart valve replacement surgery? ___ ___ ___/___
   If yes, what heart valve was replaced?
   __ mitral __ aortic

7. Have you ever had any sort of heart surgery? ___ ___ ___/___

   If yes, what type of surgery?_____

8. Have you had cardiomyopathy? ___ ___ ___/___
9. Have you had a heart aneurysm? ___ ___ ___/___
10. Have you had heart failure? ___ ___ ___/___
11. Do you have hypertension (high blood pressure)? ___ ___ ___/___
12. Have you ever had a stroke? ___ ___ ___/___
13. Do you have diabetes mellitus? ___ ___ ___/___
14. Have you ever had respiratory problems? ___ ___ ___/___
15. Have you ever been diagnosed with asthma? ___ ___ ___/___
    If yes, is the condition controlled? ___ ___ ___/___

16. Have you ever had or been told you have any other medical problems?
    (Ex. knee problems, allergies, anemia, liver disease, kidney disease, etc.)

If yes, please explain:

_____

_____

_____

17. Has your doctor ever told you not to exercise for any reason?
    Yes ___ No ___

If yes, please explain:

_____

18. Have you ever had surgical procedures? (*Ex. tonsillectomy, kidney surgery, etc.)*

If yes, please explain, and include date of surgery:

_____

_____

19. Medications:

List medications you are taking currently or take on a regular basis:

Name of drug	Times/day	Dosage (mg)	Duration of use
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Physical Activity

1. What would you consider your current physical activity level to be? Please circle the most representative choice.

Heavy Moderate Light None

2. Are you currently on a physical activity program? Yes No

If yes, what program? _____

3. List physical activities that you normally do each week, including both cardiovascular and resistance exercises:

Activity	Duration	Times/week	Intensity
Ex. Walking	*30 min*	*3*	*Moderate*
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

4. Do you plan on starting a new physical activity program anytime in the next 2 months?
Yes No

If yes, what program do you intend to start? _____

Statement of Confidentiality

I understand that the information contained on this questionnaire is regarded as confidential, and will not be released without my prior written permission. The research center may, however, use the information for statistical and other research purposes.

Signature _____ Date ___/___/___

APPENDIX C:

MUSCLE SORENESS EVALUATION

Study: Impact of Protein Supplementation on Muscle Recovery after Exercise-Induced Muscle Damage

Subject Identification Number_____
Date_____
Time_____

Muscle Soreness Evaluation

Below is a line representing a gradual scale of muscle soreness. Please mark a vertical line on the part of the scale that best represents how sore your right leg muscles feel **at this moment**:

_____

Not at all Sore — Extremely Sore

LEVEL OF EXERTION QUESTIONNAIRE

Study: Impact of Protein Supplementation on Muscle Recovery after Exercise-Induced Muscle Damage

Subject Identification Number_____
Date_____
Time_____

Level of Exertion Questionnaire

Below is a line representing a gradual scale of your level of exertion. Please mark a vertical line on the part of the scale that best represents your level of exertion on the trial you just finished:

No Exertion **Maximum Exertion**

Adverse Events Health Form

STUDY: Impact of Protein Supplementation on Muscle Recovery after Exercise-Induced Muscle Soreness

Subject ID_____ NAME: _____

Day 2 of trial, DATE: _____-____-_____

Have you had any adverse events in the last 24 hours? (circle)

Yes No

If yes, what were the events?_____

What action did you take?_____

What is the outcome (resolved, still present, etc.)?_____

Day 3 of trial, DATE: _____-____-_____

Have you had any adverse events in the last 24 hours? (circle)

Yes No

If yes, what were the events?_____

What action did you take?_____

What is the outcome (resolved, still present, etc.)?_____

APPENDIX D: RAW DATA

Table 1. Subject Height, Weight, and CPK Activity Data

Subject #	Trial	Treatment	Height (cm)	Weight (kg)			CPK activity (U/L)			
			Day 1	Day 1	Day 2	Day 3	0 hours	0.5 hours	24 hours	48 hours
ED402	1	Protein	178	74.5	71.8	71.9	52.4	58	229.4	159.9
ED402	2	Carbohydrate	178.3	72.4	72.8	72.8	76.1	72	178.6	105.8
ED402	3	Placebo	177.9	72.5	72.4	71.7	75.1	87.5	136.6	139.4
ED404	1	Carbohydrate	186.8	83.2	83.5	83.9	210.8	231.4	455.7	315.6
ED404	2	Placebo	186.7	82.5	82.2	82.7	178.3	181.1	526.4	324.3
ED404	3	Protein	187.2	82.2	81.7	80.9	149.5	NA	258.4	333.1
ED405	1	Carbohydrate	186.5	80	79.9	79.9	122.1	112.2	223.3	315.8
ED405	2	Placebo	186.7	78.5	79.1	78.9	77.5	79.3	102.8	91.5
ED405	3	Protein	187	78.2	77.5	78	199.2	231.7	203.2	119.3
ED406	1	Protein	181.3	83.8	83	82.6	148.2	169.1	486	20177.1
ED406	2	Carbohydrate	181.4	82.8	83	83.8	233	245.2	249.3	531.2
ED406	3	Placebo	181	84.4	85.1	85.2	197.3	196.1	279.3	263.8
ED407	1	Carbohydrate	176.8	75.9	76.1	76.7	72.5	60.3	250.5	143.7
ED407	2	Placebo	176.3	78.7	79	78.7	178	185.1	271.3	221.5
ED407	3	Protein	176	78.7	77.9	79.1	72.3	79.3	189.2	126.2
ED409	1	Placebo	174.5	84.5	85	84.6	168.8	176.5	923.5	758.9
ED409	2	Carbohydrate	175.7	85.5	84.3	85	289.9	286.3	1171.6	844.3
ED409	3	Protein	175.3	86.1	86.2	87.1	463.4	477.6	814.8	436.7
ED410	1	Placebo	180	71.5	70.9	71.8	66.6	75.5	169.4	122.4
ED410	2	Protein	180.2	69.9	70.2	70.4	64.4	68.9	192	124.3
ED410	3	Carbohydrate	179.6	68.7	70.9	70.8	131.2	151.3	115.5	119.5
ED411	1	Placebo	176.9	94.4	92.8	92.8	109.8	114.2	138.3	2241.3
ED411	2	Protein	177	93.6	93.7	93.1	73.3	71.4	85.5	78.8
ED411	3	Carbohydrate	177.5	92.8	93.3	94.3	69.4	70.4	179.5	733.2
ED412	1	Carbohydrate	184.5	78.4	76.9	77.2	123.8	120.3	188	159.4
ED412	2	Protein	185	79.9	79	79	160.2	159.2	240.4	246.2
ED412	3	Placebo	184	75.6	75.4	74.8	84	85.9	117.8	95.3
ED413	1	Placebo	180.6	96.8	95.6	95.7	112.4	117.7	160.7	137.6
ED413	2	Protein	179.8	96.5	95.4	95.1	100.9	100.7	92.1	96.9
ED413	3	Carbohydrate	179.6	96	96	94.5	118	128.2	111.1	106.8

Table 1. Subject Height, Weight, and CPK Activity Data (continued)

Subject #	Trial	Treatment	Height (cm)	Weight (kg)			CPK activity (U/L)			
			Day 1	Day 1	Day 2	Day 3	0 hours	0.5 hours	24 hours	48 hours
ED414	1	Placebo	184.5	94.7	94.5	95.2	158.5	166	390.1	255.9
ED414	2	Carbohydrate	186.4	93.4	93.2	94	136.3	NA	257.7	194
ED414	3	Protein	184.5	92	91.6	91.9	174.2	172.9	388.9	229.4
ED416	1	Protein	176	77.2	77.4	77.3	279.5	316.9	590	384.3
ED416	2	Carbohydrate	175.9	76.8	75.7	76.3	138.9	NA	434.6	313.8
ED416	3	Placebo	176.1	65.4	76.2	76.4	327.3	168.1	391.4	327.3
ED418	1	Placebo	170.5	62.9	62	62.4	150.1	137.1	826.8	457.8
ED418	2	Carbohydrate	170.1	62.9	62.6	62.7	158.2	159.9	205.2	130.9
ED418	3	Protein	170.2	62	61.8	62.5	116	129.5	836.4	490.9
ED419	1	Placebo	176.5	78.9	78.4	78.1	104	98.9	123.8	108.6
ED419	2	Protein	176.7	76.9	77.4	77.6	182.8	172.2	190.8	157.9
ED419	3	Carbohydrate	176.4	77.7	77.9	77.8	140.4	131	181.4	228.9
ED420	1	Protein	181.5	76.8	76.2	76.7	137.6	147.7	532.1	6737.2
ED420	2	Carbohydrate	181.6	76.6	77	78.4	239.8	238.8	271.3	224
ED420	3	Placebo	182.5	76.7	77.6	76.4	162.2	171.3	234.7	207.3
ED424	1	Placebo	192	73.2	74	74.4	101.4	94.9	158.7	170.4
ED424	2	Carbohydrate	191	72.8	73.1	72.8	138.8	154.7	240.1	147.3
ED424	3	Protein	192	72.2	73	72.4	108.6	108.4	287.1	385.8
ED425	1	Placebo	177	77.1	76.9	77.4	40	41.9	250.5	NA
ED425	2	Carbohydrate	177.3	78.9	78.8	78.3	53.4	39.9	156.9	111.4
ED425	3	Protein	178.5	79	79.3	78.9	46	47.6	88.8	56.7
ED426	1	Placebo	170.9	69	69.4	69.4	107.1	113.9	393.5	939.5
ED426	2	Carbohydrate	170.9	69	68.5	69	111.9	117.2	239	216.7
ED426	3	Protein	171.3	68.2	68.9	69	95.3	104	183.9	120.6
ED427	1	Protein	184	98.9	97.8	98.4	147.8	152.1	393.9	572.4
ED427	2	Carbohydrate	184.2	99.1	99.3	98	121.3	128.2	228.1	176.2
ED427	3	Placebo	184	99.8	99.8	99	478.6	469.8	422.2	234.2
ED428	1	Carbohydrate	184.3	78.5	78.6	78.7	265.2	290.4	283.5	269.6
ED428	2	Protein	186.1	78.4	78.6	78.2	170	184.1	172.5	174.2
ED428	3	Placebo	185	79.9	78.9	78.3	165	154.7	278	184.6
ED429	1	Placebo	170.7	67.2	66.2	66.6	172.5	184.1	224.3	160.4
ED429	2	Carbohydrate	171.7	65.4	65.7	65.9	111.4	123.1	123.4	92.9
ED429	3	Protein	171	65.2	64.2	65.4	129.5	137.4	153.6	107.6

Table 2. Subject Perceived Muscle Soreness and Level of Exertion Data

Subject #	Trial	Treatment	Muscle Soreness (0-10 cm scale)						Level of Exertion (0-10 cm scale)				
			1	2	3	4	5	6	1	2	3	4	5
ED402	1	Protein	1.3	4.6	6.7	8.6	6.3	7.3	9.5	8.6	9.6	9.3	9.3
ED402	2	Carbohydrate	1.2	8.3	3.4	5.9	2.3	2.6	8.2	9.4	9.6	8.8	7.2
ED402	3	Placebo	3	7	5.1	8.7	5.9	5.3	8	8.7	9	7.3	8.2
ED404	1	Carbohydrate	0.4	6.5	7.5	9.1	8.7	7.2	8.7	6.5	9.6	1.9	2.5
ED404	2	Placebo	0.3	0.7	3.2	4.9	3.7	4.7	8.5	7.9	9.1	5.6	5.2
ED404	3	Protein	0.3	3.5	1.3	3.5	3.7	6.2	4.4	7.4	9.3	4.9	3.7
ED405	1	Carbohydrate	0.2	7.8	2.7	4.5	3.4	5.7	8.7	8.9	9.2	7.7	7.8
ED405	2	Placebo	0.4	8.2	1.6	3.8	2.3	4.9	8.7	9.1	9.1	9.4	9.3
ED405	3	Protein	0.6	8.9	1.8	3	0.9	3.4	8	9	8.8	9.2	9.3
ED406	1	Protein	1.7	9	5.9	6.5	7.2	6.4	4.4	5.7	8.9	4.2	5.1
ED406	2	Carbohydrate	1.7	7.3	5.4	6.1	5.7	5.9	3.9	6	7.4	4.9	3.2
ED406	3	Placebo	1.1	7	3.7	5.8	3.1	5.3	4.6	5.7	6.9	4.9	5.5
ED407	1	Carbohydrate	1.1	3.2	3.4	4.5	4.8	5	8	7.7	8.1	8.4	8.9
ED407	2	Placebo	0.4	7.5	1.8	3.8	2.5	3.9	7.8	9.5	9.5	8.7	9.4
ED407	3	Protein	0.7	7.5	2.7	6.2	2.3	4.1	9.9	8.3	8.3	8.9	9
ED409	1	Placebo	0.7	10	5	4.8	2.4	2.4	9.8	9.8	9.8	9.8	9.8
ED409	2	Carbohydrate	0	4.7	2.7	3.7	2	3.1	9.8	9.7	9.8	9.8	9.8
ED409	3	Protein	1	4.8	2.9	4.3	1.3	1.9	9.8	9.8	9.7	9.7	9.7
ED410	1	Placebo	0.5	1.9	2.4	2.9	2.7	3.1	6	7.5	8	7.8	8
ED410	2	Protein	1.8	5.8	2.6	3.1	2.4	2.5	7.8	7.8	7.8	8	6.7
ED410	3	Carbohydrate	1.5	2.6	1.8	2	2.4	2.5	6.5	7.2	7.5	7.9	7.5
ED411	1	Placebo	1.5	8.1	5.8	7.2	7.8	8.3	6.9	8.7	8.6	8.2	7.8
ED411	2	Protein	1.1	5.2	3.2	3.6	2.1	2.2	7.5	9	8.5	8.5	8.7
ED411	3	Carbohydrate	2.7	7.6	9.3	9.3	8.3	7.6	8.3	8.6	9.3	8.8	9
ED412	1	Carbohydrate	0.2	7.9	1.4	4.6	1.9	3.9	1.9	3.2	7.9	2.6	4.3
ED412	2	Protein	0.4	7.8	2.2	3.2	0.7	1	3	3.9	8.2	2.6	2.1
ED412	3	Placebo	0	6	1.3	3.1	1	1.8	1.9	2.8	8.4	2.1	2.7
ED413	1	Placebo	0.1	6.2	8	8.2	5.3	2.8	7.1	8	9.1	5.5	6.6
ED413	2	Protein	1.6	3.6	0.9	2	0	0.6	6.2	7.4	8.7	7.4	7.8
ED413	3	Carbohydrate	0.1	3.2	2	3.1	1.7	2.1	7.1	8.8	8.5	7.1	9

Table 2. Subject Perceived Muscle Soreness and Level of Exertion Data (continued)

Subject #	Trial	Treatment	Muscle Soreness (0-10 cm scale)						Level of Exertion (0-10 cm scale)				
			1	2	3	4	5	6	1	2	3	4	5
ED414	1	Placebo	0	5.4	3.9	6.2	5	7	8.2	8.6	9	8.4	8.3
ED414	2	Carbohydrate	2.3	5.6	3	4.7	3.7	5.7	8.3	8	8.8	7.9	7.8
ED414	3	Protein	1.8	5.3	3.4	3.3	1.8	5.4	7.1	7.3	8.4	8	8.4
ED416	1	Protein	1.4	NA	2.3	4	3	3.9	8	8.3	8.5	8.7	8.7
ED416	2	Carbohydrate	0.5	7.4	3.6	4.5	3.4	4	8.9	7.7	8.3	8.8	8.5
ED416	3	Placebo	2.5	2.4	2.4	3.7	3.7	4.4	8.9	8.4	8.5	8.5	8.9
ED418	1	Placebo	1.2	4.5	7.5	8.5	8.5	6.2	9	9.2	7	8.5	7.7
ED418	2	Carbohydrate	1.2	5.9	5.7	7.1	4	5.2	8	7.7	9.1	7.8	7.7
ED418	3	Protein	2.3	5.9	7	8.4	6.4	7	8	8.9	9	8.3	8.3
ED419	1	Placebo	1.3	2.4	5.8	5.5	6.2	6.9	7.5	6.4	6.5	3.9	5.8
ED419	2	Protein	1.2	3.7	5.3	5.7	6.1	6.6	7.4	7.6	8.7	6	6.1
ED419	3	Carbohydrate	1.6	4.1	3.1	4.6	2.9	3.4	6	5.8	6.6	4.8	5.2
ED420	1	Protein	0.2	6.7	5.6	5.6	9.9	9.6	8.7	8.9	9.7	7.7	9
ED420	2	Carbohydrate	0.4	0.7	0.8	0.7	1.7	1.6	8.9	9.3	9.5	9.1	9.1
ED420	3	Placebo	0.4	0.5	1.2	1.1	2	1	9.5	9.3	9.5	9.3	9
ED424	1	Placebo	0.1	2.6	2.5	3.5	4.7	6.2	7.3	9.4	9.3	7.2	7
ED424	2	Carbohydrate	0.4	4.9	1.5	1.7	2.9	4.1	7.1	8.6	8.7	7.6	8.5
ED424	3	Protein	0	4.2	5.8	7.8	7.6	9	7.8	8.6	8.9	9	8.9
ED425	1	Placebo	0.2	4.8	6.4	7	8.7	9.3	6.4	8.3	9.2	4.1	6.1
ED425	2	Carbohydrate	0.2	1.6	2	3.5	4	4.4	5.8	6.7	8.8	5.6	5.8
ED425	3	Protein	0.1	0.3	2.5	2.2	2.4	2.1	5.4	6.5	8.8	6.2	6.4
ED426	1	Placebo	0.2	6.7	2.8	2.5	3.1	2.7	8.4	8.7	8.5	8.9	8.5
ED426	2	Carbohydrate	1.2	8.6	2.8	3	2.2	2.4	7.8	8.8	8.9	8.4	9.1
ED426	3	Protein	0.5	7.1	1.1	0.4	0.5	0.5	9.2	8.8	9.2	9.4	9.5
ED427	1	Protein	2	4.9	5.6	5	8	6.6	7	8.5	9	8.2	8
ED427	2	Carbohydrate	1.7	8	5.4	4.3	2.7	1.6	7.6	8	8.1	8.4	9.2
ED427	3	Placebo	4.6	9	7	6.2	4.4	2.5	7.9	9	8.6	8.6	8.6
ED428	1	Carbohydrate	0.5	1.6	7.2	5.1	6.2	5.7	8.6	9.5	9.7	8.9	8.9
ED428	2	Protein	0.2	2.3	6.4	8	4	6.3	8.9	9.5	9.5	8.9	9.5
ED428	3	Placebo	0.2	3	4.8	5.6	6.5	6.4	9.2	9.1	8.3	9.6	9.5
ED429	1	Placebo	1.9	6.6	5.3	7	6.8	6.5	6.7	8.4	8.6	6.7	6.6
ED429	2	Carbohydrate	2.3	7	4.8	4.4	7.9	6.5	7.2	7.8	8.9	7.2	7.9
ED429	3	Protein	0.5	6.5	2.6	4	3	4.8	6.4	8.5	7.6	6.8	7.1

Table 3. Subject Blood Pressure and Heart Rate Data

Subject #	Trial	Treatment	Blood Pressure (Systolic/Diastolic)				Heart Rate (beats/min)			
			0 hr	1 hr	24 hr	48 hr	0 hr	1 hr	24 hr	48 hr
ED402	1	Protein	129/74	118/77	119/85	115/72	84	73	95	95
ED402	2	Carbohydrate	128/70	131/78	131/74	120/69	92	71	95	79
ED402	3	Placebo	124/82	116/82	126/81	113/65	84	68	76	70
ED404	1	Carbohydrate	126/92	120/82	126/80	126/88	56	96	66	72
ED404	2	Placebo	125/78	142/81	140/76	134/83	75	80	71	64
ED404	3	Protein	129/73	139/85	124/80	125/76	66	84	70	65
ED405	1	Carbohydrate	145/80	138/82	122/73	124/74	84	89	83	74
ED405	2	Placebo	139/84	127/77	128/77	131/76	85	84	86	84
ED405	3	Protein	127/72	127/79	126/70	142/78	79	91	71	89
ED406	1	Protein	118/68	131/85	135/70	123/72	51	60	51	47
ED406	2	Carbohydrate	124/71	128/81	134/78	123/69	54	55	62	58
ED406	3	Placebo	117/72	115/71	127/68	122/65	58	55	47	47
ED407	1	Carbohydrate	143/83	153/86	142/63	141/86	56	60	58	66
ED407	2	Placebo	143/83	128/48	127/76	133/76	67	62	60	58
ED407	3	Protein	149/90	137/84	144/82	134/82	76	72	61	58
ED409	1	Placebo	129/67	135/80	126/69	128/74	65	72	61	67
ED409	2	Carbohydrate	118/95	135/81	135/61	130/70	64	71	62	68
ED409	3	Protein	145/65	130/81	126/62	118/67	71	71	69	65
ED410	1	Placebo	124/65	124/67	104/60	128/64	63	66	73	67
ED410	2	Protein	125/60	122/64	125/50	114/53	68	58	58	64
ED410	3	Carbohydrate	120/77	116/69	118/61	110/61	55	51	63	58
ED411	1	Placebo	158/65	139/63	137/67	120/76	67	64	69	63
ED411	2	Protein	146/74	141/45	147/79	140/73	62	68	58	67
ED411	3	Carbohydrate	146/74	157/89	150/77	144/84	67	88	68	68
ED412	1	Carbohydrate	140/71	146/74	138/68	147/78	54	74	72	72
ED412	2	Protein	144/71	141/71	136/74	139/65	56	67	64	51
ED412	3	Placebo	131/107	128/83	143/73	128/82	84	75	83	96
ED413	1	Placebo	129/76	118/72	138/74	131/71	61	72	71	74
ED413	2	Protein	116/72	104/67	129/73	130/74	60	61	75	74
ED413	3	Carbohydrate	130/73	115/89	128/80	124/83	73	71	81	80

Table 3. Subject Blood Pressure and Heart Rate Data (continued)

Subject #	Trial	Treatment	Blood Pressure (Systolic/Diastolic)				Heart Rate (beats/min)			
			0 hr	1 hr	24 hr	48 hr	0 hr	1 hr	24 hr	48 hr
ED414	1	Placebo	140/71	147/72	149/68	134/79	53	47	44	45
ED414	2	Carbohydrate	134/64	156/82	144/75	129/70	46	64	44	44
ED414	3	Protein	149/66	122/51	134/75	126/78	50	54	53	52
ED416	1	Protein	130/77	119/68	114/73	123/59	61	53	50	48
ED416	2	Carbohydrate	113/64	120/64	122/61	119/61	55	54	62	45
ED416	3	Placebo	112/66	131/65	118/59	127/65	48	60	45	52
ED418	1	Placebo	128/67	133/70	130/79	126/69	54	80	80	61
ED418	2	Carbohydrate	125/68	128/87	80/58	110/58	60	80	66	73
ED418	3	Protein	118/79	108/76	126/68	131/68	80	76	75	66
ED419	1	Placebo	126/81	126/81	119/78	130/79	52	54	51	66
ED419	2	Protein	124/78	122/75	124/80	136/77	50	54	52	46
ED419	3	Carbohydrate	135/78	132/71	133/76	130/73	60	61	65	79
ED420	1	Protein	100/61	123/71	131/72	119/68	69	73	73	80
ED420	2	Carbohydrate	114/79	118/80	137/74	103/63	68	80	85	81
ED420	3	Placebo	118/63	106/57	105/65	141/69	82	74	58	84
ED424	1	Placebo	123/64	118/63	104/63	116/64	59	75	64	71
ED424	2	Carbohydrate	111/65	109/64	118/66	121/70	62	62	57	63
ED424	3	Protein	115/68	119/70	118/68	122/71	69	79	61	63
ED425	1	Placebo	144/72	122/66	123/70	125/64	59	57	59	64
ED425	2	Carbohydrate	135/73	131/78	129/70	136/72	65	73	61	68
ED425	3	Protein	123/68	113/70	129/67	145/72	66	64	70	69
ED426	1	Placebo	151/75	127/72	159/94	144/77	76	60	91	73
ED426	2	Carbohydrate	140/84	123/75	152/91	161/68	76	67	89	78
ED426	3	Protein	148/86	138/80	154/77	127/74	96	88	79	83
ED427	1	Protein	145/81	142/82	137/87	130/84	86	83	83	80
ED427	2	Carbohydrate	140/74	142/77	143/79	157/85	85	79	82	82
ED427	3	Placebo	149/86	146/94	131/78	144/86	95	93	96	81
ED428	1	Carbohydrate	131/62	144/70	125/66	111/65	64	73	71	60
ED428	2	Protein	122/59	127/65	120/58	111/62	59	71	60	54
ED428	3	Placebo	106/55	128/69	120/60	131/69	67	73	75	87
ED429	1	Placebo	128/68	125/68	126/62	126/62	71	69	64	55
ED429	2	Carbohydrate	136/71	137/70	135/66	139/66	92	74	74	64
ED429	3	Protein	122/68	110/64	128/70	127/58	62	64	66	63

Table 4. Subject Dietary Intake Data from 24-hour Records

Subject #	Trial	Treatment	Avg. Total Energy (kcal/day)	Avg. Total Protein (g/day)	Total Carbohydrate (g/day)	Total Fat (g/day)
ED402	1	Protein	2152	65.829	228.44	67.49
ED402	2	Carbohydrate	1721	57.71	211.62	75.21
ED402	3	Placebo	2029	70.61	243.71	86.5
ED404	1	Carbohydrate	2648	99.21	344.38	96.88
ED404	2	Placebo	2486	107.6	284.46	98.26
ED404	3	Protein	2536	95.75	318.14	94.56
ED405	1	Carbohydrate	2391	76.74	326.04	90.1
ED405	2	Placebo	2140	58.64	332.8	66.14
ED405	3	Protein	2293	77.13	298.2	94.17
ED406	1	Protein	2516	94.56	314.91	100.32
ED406	2	Carbohydrate	1945	68.54	277.98	66.88
ED406	3	Placebo	2118	89.97	247.6	89.09
ED407	1	Carbohydrate	3568	108.39	497.5	126.41
ED407	2	Placebo	2830	87.87	344.15	124.64
ED407	3	Protein	2088	71.2	279.35	80.06
ED409	1	Placebo	1301	83.3	219.25	13.29
ED409	2	Carbohydrate	1793	140.01	264.36	77.24
ED409	3	Protein	1269	59.93	220.57	21.16
ED410	1	Placebo	1673	85.71	177.51	70.91
ED410	2	Protein	1822	95.27	190.97	202.25
ED410	3	Carbohydrate	1619	61.55	218.03	57.41
ED411	1	Placebo	1724	61.3	246.95	55.5
ED411	2	Protein	2389	50.49	205.59	63.75
ED411	3	Carbohydrate	1228	89.81	91.29	55.46
ED412	1	Carbohydrate	2223	93.55	229.1	98.59
ED412	2	Protein	1961	72	272.06	66.76
ED412	3	Placebo	1700	59.98	248.54	56.33
ED413	1	Placebo	1364	92.97	104.48	53.74
ED413	2	Protein	1322	66.48	160.01	48.85
ED413	3	Carbohydrate	924	98.29	89.43	20.58

Table 4. Subject Dietary Intake Data from 24-hour Records (continued)

Subject #	Trial	Treatment	Avg. Total Energy (kcal/day)	Avg. Total Protein (g/day)	Avg. Total Carbohydrate (g/day)	Avg. Total Fat (g/day)
ED414	1	Placebo	2021	62.96	232.87	76.86
ED414	2	Carbohydrate	2176	96.5	234.5	71.58
ED414	3	Protein	1421	70.94	123.69	62.64
ED416	1	Protein	1673	90.23	228.19	49.42
ED416	2	Carbohydrate	1815	94.64	276.33	39.91
ED416	3	Placebo	1444	108.71	157.68	44.65
ED418	1	Placebo	2054	95.22	210.29	96.69
ED418	2	Carbohydrate	1735	63.49	215.65	71.55
ED418	3	Protein	2209	97.2	252.04	88.6
ED419	1	Placebo	2229	84.3	282.16	87.16
ED419	2	Protein	1738	54.18	256.59	58.57
ED419	3	Carbohydrate	2172	66.54	261.36	101.03
ED420	1	Protein	1610	53.63	213.11	48.12
ED420	2	Carbohydrate	2318	71.16	310.1	94.29
ED420	3	Placebo	1829	62.82	228.12	81.26
ED424	1	Placebo	2366	81.68	304.92	97
ED424	2	Carbohydrate	2607	87.32	365.04	96.83
ED424	3	Protein	1665	66.02	247.68	48.01
ED425	1	Placebo	1581	61.16	194.4	63.54
ED425	2	Carbohydrate	1168	44.44	150.93	130.39
ED425	3	Protein	1228	50.68	179.23	36.62
ED426	1	Placebo	1949	61.68	320.5	51.72
ED426	2	Carbohydrate	1768	85.09	239.27	59.17
ED426	3	Protein	2193	64.6	316.65	77.93
ED427	1	Protein	2613	105.39	333.41	100.74
ED427	2	Carbohydrate	2039	77.58	217.93	94.51
ED427	3	Placebo	1994	95.54	174.68	103.38
ED428	1	Carbohydrate	1658	67.96	200.15	68.08
ED428	2	Protein	1944	92.04	205.11	86.16
ED428	3	Placebo	964	48.1	116.9	34.56
ED429	1	Placebo	2042	83.16	281.09	74.11
ED429	2	Carbohydrate	1970	86.14	177.49	100.92
ED429	3	Protein	1953	78.37	273.28	65.26

Table 5. Subject Physical Activity Data from 24-hour Records

Subject #	Trial	Treatment	Avg. Rest (minutes/day)	Avg. Light Exercise (minutes/day)	Avg. Moderate Exercise (minutes/day)	Avg. Heavy Exercise (minutes/day)
ED402	1	Protein	1366.7	66.7	6.7	0.0
ED402	2	Carbohydrate	1345.0	95.0	0.0	0.0
ED402	3	Placebo	1388.3	31.7	20.0	0.0
ED404	1	Carbohydrate	1396.7	23.3	0.0	20.0
ED404	2	Placebo	1410.0	0.0	0.0	30.0
ED404	3	Protein	1403.3	6.7	0.0	30.0
ED405	1	Carbohydrate	1389.3	50.7	0.0	0.0
ED405	2	Placebo	1416.3	23.7	0.0	0.0
ED405	3	Protein	1389.0	51.0	0.0	0.0
ED406	1	Protein	1156.7	243.3	26.7	13.3
ED406	2	Carbohydrate	1100.0	100.0	240.0	0.0
ED406	3	Placebo	1160.0	240.0	40.0	0.0
ED407	1	Carbohydrate	1391.0	48.3	0.7	0.0
ED407	2	Placebo	1375.7	61.7	2.7	0.0
ED407	3	Protein	1409.3	30.0	0.7	0.0
ED409	1	Placebo	1095.0	345.0	0.0	0.0
ED409	2	Carbohydrate	1080.0	360.0	0.0	0.0
ED409	3	Protein	1403.3	10.0	10.0	16.7
ED410	1	Placebo	1415.0	25.0	0.0	0.0
ED410	2	Protein	1405.0	35.0	0.0	0.0
ED410	3	Carbohydrate	840.0	300.0	300.0	0.0
ED411	1	Placebo	1353.3	55.0	31.7	0.0
ED411	2	Protein	1176.7	216.7	46.7	0.0
ED411	3	Carbohydrate	1338.3	65.0	36.7	0.0
ED412	1	Carbohydrate	1346.7	53.3	40.0	0.0
ED412	2	Protein	1358.3	60.0	21.7	0.0
ED412	3	Placebo	1430.0	3.3	6.7	0.0
ED413	1	Placebo	1260.0	180.0	0.0	0.0
ED413	2	Protein	1360.0	80.0	0.0	0.0
ED413	3	Carbohydrate	1280.0	160.0	0.0	0.0

Table 5. Subject Physical Activity Data from 24-hour Records (continued)

Subject #	Trial	Treatment	Avg. Rest (minutes/day)	Avg. Light Exercise (minutes/day)	Avg. Moderate Exercise (minutes/day)	Avg. Heavy Exercise (minutes/day)
ED414	1	Placebo	1296.7	15.0	128.3	0.0
ED414	2	Carbohydrate	1391.7	15.0	33.3	0.0
ED414	3	Protein	1395.0	20.0	25.0	0.0
ED416	1	Protein	1090.0	245.0	96.7	8.3
ED416	2	Carbohydrate	1283.3	76.7	80.0	0.0
ED416	3	Placebo	1111.7	198.3	121.7	8.3
ED418	1	Placebo	1390.0	0.0	50.0	0.0
ED418	2	Carbohydrate	1400.0	40.0	0.0	0.0
ED418	3	Protein	1240.0	0.0	200.0	0.0
ED419	1	Placebo	1411.7	25.0	3.3	0.0
ED419	2	Protein	1378.3	46.7	15.0	0.0
ED419	3	Carbohydrate	1343.3	50.0	46.7	0.0
ED420	1	Protein	1366.7	73.3	0.0	0.0
ED420	2	Carbohydrate	1328.3	101.7	10.0	0.0
ED420	3	Placebo	1371.7	68.3	0.0	0.0
ED424	1	Placebo	1340.0	40.0	60.0	0.0
ED424	2	Carbohydrate	1305.0	135.0	0.0	0.0
ED424	3	Protein	1425.0	15.0	0.0	0.0
ED425	1	Placebo	1336.7	103.3	0.0	0.0
ED425	2	Carbohydrate	1291.7	148.3	0.0	0.0
ED425	3	Protein	1302.7	133.3	4.0	0.0
ED426	1	Placebo	1193.3	246.7	0.0	0.0
ED426	2	Carbohydrate	1355.0	85.0	0.0	0.0
ED426	3	Protein	1383.3	56.7	0.0	0.0
ED427	1	Protein	1390.0	50.0	0.0	0.0
ED427	2	Carbohydrate	1400.0	40.0	0.0	0.0
ED427	3	Placebo	1360.0	80.0	0.0	0.0
ED428	1	Carbohydrate	1200.0	205.0	35.0	0.0
ED428	2	Protein	1250.0	190.0	0.0	0.0
ED428	3	Placebo	1235.0	205.0	0.0	0.0
ED429	1	Placebo	1351.7	43.3	45.0	0.0
ED429	2	Carbohydrate	1353.3	40.0	20.0	26.7
ED429	3	Protein	1336.7	30.0	33.3	40.0

Table 6. Subject Biodex Isokinetic Dynamometer Muscle Strength Indices

Subject #, (Trial)	Extension 60deg				Flexion 60deg			
ED402(1)	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque
Day 1	97.8	79.3	76.1	90.2	65.7	57.2	50.5	58.5
Day 2	116.6	84.5	80.8	105.6	49.4	39.6	38.9	45.7
Day 3	137.7	104	93.4	120.8	52.4	45.1	37.7	49.9
ED402(2)								
Day 1	138.1	103.8	106.6	134.6	67.1	55	59.8	64.4
Day 2	139.1	94.3	86.3	119.2	60.9	52.5	50.8	58.4
Day 3	141.3	102.9	99.4	132.9	69.5	64.6	56.5	63.8
ED402(3)								
Day 1	127.6	95.3	91.5	122.3	59	53.8	48.2	58.5
Day 2	126.6	100.8	91.4	117.2	63.3	66.6	49.2	59.6
Day 3	134.7	106.5	94.2	119	62.3	63.3	54.8	56.8
ED404(1)								
Day 1	160.1	114.6	117.4	145.6	108.3	93.4	7.7	97.5
Day 2	151.1	93.7	102.7	139.6	84.3	60.8	65.5	81.7
Day 3	152.1	88.2	97.4	140.2	66.2	38.6	45.5	61.1
ED404(2)								
Day 1	143.2	113.3	110	137.4	95.7	95.5	89.3	89.7
Day 2	138.2	98.1	107	127.4	91.9	74.4	91.2	85
Day 3	139.9	77	95.9	126.8	62.8	43.6	55.6	59.9
ED404(3)								
Day 1	159	116.3	120.1	144	92.7	87.8	89.1	89.7
Day 2	142.1	82.2	91.3	126.8	77.3	59.8	63.1	69.7
Day 3	161.6	108.3	121.1	142.3	80.8	57.7	68.2	71.1
ED405(1)								
Day 1	132.5	112.6	104.8	112.8	73.7	69.9	65.7	64.9
Day 2	130.1	111.2	101.5	113.3	77.5	64.8	54.7	66
Day 3	139.6	106.7	96.3	113.7	56	48.3	45.3	53.2
ED405(2)								
Day 1	128.5	101.3	102.1	113.3	72.2	59.6	63.4	67.5
Day 2	135.2	106.7	96.8	110	61.9	55.2	55.5	56.2
Day 3	144.6	118.8	111.9	124.8	66.9	61.3	60.1	59.5
ED405(3)								
Day 1	130.8	107.5	108	117.5	67.5	60	55.4	59.6
Day 2	131.5	115.1	113.8	123	65.1	56.5	57.7	60.8
Day 3	141.3	111.1	118.2	130.1	80.9	68	68.8	70.2

Table 6. Subject Biodex Isokinetic Dynamometer Muscle Strength Indices (continued)

Subject #, (Trial)	Extension 60deg				Flexion 60deg			
ED406(1)	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque
Day 1	87.2	75.4	67.4	78.4	43.9	38.5	31.4	38.5
Day 2	93.8	81.1	71.9	89.5	40	45	38.7	38.2
Day 3	79.7	75.4	65.5	77.2	32.6	35.5	60.5	31.1
ED406(2)								
Day 1	79.1	81	63.9	72.5	46.7	56.7	43	42.1
Day 2	66.4	60.1	53.4	63.6	34.2	38.4	60.9	33
Day 3	62.7	56.3	47.4	57.1	33.4	33.7	28.5	30.2
ED406(3)								
Day 1	54.4	56.2	45.2	50.4	31.9	38.3	28.8	28.2
Day 2	52.4	54.3	41.3	46.6	29.2	32.6	25.5	28
Day 3	52	46.7	39.8	45.2	31	32.2	27.8	28.8
ED407(1)								
Day 1	118.5	96.8	73.3	90.3	56.4	67.2	54.3	54
Day 2	101.8	92.9	76.1	94.7	52	54.5	44.4	46.9
Day 3	140.6	150.8	99.8	130.9	62	72.2	48.8	56.8
ED407(2)								
Day 1	119.9	106.5	81.9	102.2	85.9	100.4	81	79.3
Day 2	121.5	107.3	85.8	112.2	74.3	85.7	65.8	68.6
Day 3	138.8	127.5	102.3	126.6	73.1	90.2	67.7	68.1
ED407(3)								
Day 1	139.3	124.7	98.6	127	74.2	91.9	71.5	68.8
Day 2	134.3	117.2	91.1	124.6	76	93.9	75.3	71.6
Day 3	152.9	128.1	9.3	131.6	95.6	121	94.8	87.8
ED409(1)								
Day 1	151.6	97.7	116.6	130.2	75.8	59.3	69.8	70.1
Day 2	131.2	94.3	93.6	109.7	66.2	59.2	54.3	59.1
Day 3	119.5	101	100.9	112.8	64.1	57.1	60.4	57.6
ED409(2)								
Day 1	138.7	111.2	123.4	134.5	76.7	69	76.1	71.2
Day 2	139.7	110.2	114	125.4	70.9	60.2	74.5	65.1
Day 3	147.6	102	128.3	136.5	80	57.9	74.3	72.8
ED409(3)								
Day 1	133.8	94.3	99.8	104.6	74.7	50.4	66.1	64.8
Day 2	148.7	109.7	106.2	115.5	77.9	63	67.9	70.3
Day 3	135.7	96.9	109.1	118.2	80	57.6	73.8	74.9

Table 6. Subject Biodex Isokinetic Dynamometer Muscle Strength Indices (continued)

Subject #, (Trial)	Extension 60deg				Flexion 60deg			
ED410(1)	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque
Day 1	129.1	141.3	91.6	114.1	72.4	104.8	71.2	69.3
Day 2	122	108.8	88.3	110.9	75.8	89	66.3	68.8
Day 3	122.7	114.6	87.1	113.5	69.9	85.6	56	61.1
ED410(2)								
Day 1	122.9	110.1	85.7	115.4	69.8	91.1	70.7	66.8
Day 2	127.5	113.4	90.2	122	66.4	79.9	60.7	58.3
Day 3	127.3	112.8	95	117.8	64.1	76.8	63.8	58.4
ED410(3)								
Day 1	117.9	100.7	88.2	106.5	80.1	86.2	71.5	68.7
Day 2	129.1	109.8	103.4	115.8	69.6	62.5	66.3	65.8
Day 3	145.5	136	114.2	129.6	79.6	97.5	85.3	78.1
ED411(1)								
Day 1	119.9	98.5	115.3	116.1	79.6	75.1	85.7	77.2
Day 2	113.4	85.2	105	108.3	66.9	54.2	53.6	63.5
Day 3	131.5	106.3	120.7	124.4	62.3	55.9	63	60.2
ED411(2)								
Day 1	130.7	101.3	109.5	119.9	90.9	86.3	94.5	88.9
Day 2	127.4	87	97	114.7	86.8	71.3	80.7	78.4
Day 3	118.9	86.5	97.7	106.3	86.3	75.5	86.2	84.2
ED411(3)								
Day 1	144.8	103.9	116.5	131.3	96.5	89.8	98.5	92.2
Day 2	143	117.4	125.3	131.6	84.2	3.3	78.2	77.8
Day 3	114.2	81.8	98.8	109.4	77.8	64.5	74.6	74.7
ED412(1)								
Day 1	118.5	101.4	96.5	104.4	82.3	85.1	80	78.7
Day 2	127.4	113.9	100.7	115.5	78.5	92.2	75.6	75.9
Day 3	156.2	144.5	124.5	143.7	82	96.3	79.6	80.5
ED412(2)								
Day 1	147	147.7	111	134.2	88.2	112	69.5	78.2
Day 2	154.6	137.8	117.9	142.4	88.6	94.2	80.6	85.4
Day 3	140.3	130.1	107.1	131.1	84.9	97.7	75.8	79.6
ED412(3)								
Day 1	133.3	126.9	103.9	122.7	87.9	104.8	83.6	84
Day 2	163.2	147.6	116.3	146.5	95.4	108.1	87	92
Day 3	142.1	127.9	102.6	132.8	87.9	97.6	77.7	85.4

Table 6. Subject Biodex Isokinetic Dynamometer Muscle Strength Indices (continued)

Subject #, (Trial)	Extension 60deg				Flexion 60deg			
ED413(1)	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque
Day 1	120.8	119.3	99.1	105.2	65.3	67.8	60.7	58.2
Day 2	90.9	74.5	74.9	82.1	50.2	43.6	39.1	45.3
Day 3	107.6	77.9	73	91.7	58.1	45.6	39.8	50.7
ED413(2)								
Day 1	106	84.2	82.9	95	64.6	55.9	57.9	58.7
Day 2	111.3	83.3	88	100.2	66.8	60.2	63.3	62.4
Day 3	107.2	89.5	90.9	100.3	70.7	68.6	67.7	65.3
ED413(3)								
Day 1	111.5	83.1	82.4	96.4	66.1	62	59.9	62.7
Day 2	113.4	83.3	87.3	103.5	72.2	60	63.6	65.8
Day 3	130	120.8	110.6	117.4	80.3	78.4	70.7	77.2
ED414(1)								
Day 1	168.6	126.5	124.9	151.7	97.3	111.1	95.4	89.7
Day 2	164.3	128	123.9	150.6	91.8	100.6	91	86
Day 3	166.9	138.4	123.7	148.7	94.7	102.8	91.1	91.1
ED414(2)								
Day 1	173	151.9	145.8	162.2	109.9	128	107.6	96.7
Day 2	158.8	134.6	129.2	151.9	90.6	99.6	92	85.3
Day 3	164.3	134.6	127.8	155.1	95.5	104.7	98.2	88.6
ED414(3)								
Day 1	172.1	139.1	128.6	163.2	98.3	99.1	90.1	90.7
Day 2	149.5	113.5	103.9	141.3	92	88.3	78.5	84.3
Day 3	158	116.6	115.6	144.7	93.3	92.2	84.6	84.1
ED416(1)								
Day 1	132.8	131.1	116.3	126.2	89.6	96.8	85.1	87.5
Day 2	159.2	135.9	124.5	147	101.8	104.2	92.5	96.8
Day 3	156.7	140	128	144.3	103.7	104.1	77.4	89
ED416(2)								
Day 1	157.4	145.1	140.4	144.5	100.8	96.6	93.5	93.8
Day 2	160.6	146.1	135.3	153.6	102.6	95.6	81.3	95.2
Day 3	157.8	127.8	129.8	144.2	91.9	73.9	74.3	89
ED416(3)								
Day 1	172.9	143.4	134.9	153.5	107.7	101.7	95.5	103.9
Day 2	169.1	138.3	128.5	156.6	99.9	84.8	79.1	91.7
Day 3	157.7	131	119.5	145.6	93.3	89.5	79.7	87.2

Table 6. Subject Biodex Isokinetic Dynamometer Muscle Strength Indices (continued)

Subject #, (Trial)	Extension 60deg				Flexion 60deg			
ED418(1)	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque
Day 1	122.8	122.3	89.9	110.6	76	101	77.5	73.8
Day 2	99.1	88.8	72.5	89.7	53	63.6	50.7	51.9
Day 3	124	126.4	99.9	115.7	55.2	69.6	54.1	50.9
ED418(2)								
Day 1	108.7	97.9	50.4	96.1	64.6	76.5	62	59.8
Day 2	108.7	104	80.5	101.7	61.5	75.2	57.5	59.6
Day 3	116.2	117.4	85.7	109.8	65.6	89.6	63.3	61.7
ED418(3)								
Day 1	137.1	135.6	95.4	123.1	76.8	107.4	72.3	68.8
Day 2	122	118.6	93.7	116.4	75.1	98.1	72.7	70.1
Day 3	113	106.8	86.1	107.7	75.2	99.5	75.2	74.2
ED419(1)								
Day 1	97.9	54.3	69.3	85.7	54.4	35	43.7	48.5
Day 2	68.8	38	39.3	48.6	36.4	19.6	13.9	27.3
Day 3	103.2	62.8	67.8	81.4	48.8	30.7	38.3	43.1
ED419(2)								
Day 1	91.6	64.1	70.9	81.3	49.7	40.9	41.6	42.1
Day 2	75.2	56.4	44.3	57.6	46.4	38.2	36.9	38.8
Day 3	95.8	71.9	68.4	77.8	52.5	43.8	49.4	48.5
ED419(3)								
Day 1	120	96.1	91.2	93.8	62.7	62.4	61.9	58.1
Day 2	118.4	93.7	96.4	104.1	69.5	58.6	64.6	62.7
Day 3	117.8	83	78	100.5	61.2	52.2	52.3	55.2
ED420(1)								
Day 1	108.5	99	77.1	90.9	81.2	81.4	78.7	78.3
Day 2	90.4	74.5	67.7	77.6	50.9	81.2	45.1	46.8
Day 3	96.2	71.4	69.2	79.8	44.9	34.4	37.8	39.7
ED420(2)								
Day 1	106.1	92.5	86	98.4	77.8	82.4	71.2	71
Day 2	132.8	99.4	86.2	103.2	82.8	81.7	75.9	72.1
Day 3	142.8	123.6	103.5	120.6	93	100	85	83.4
ED420(3)								
Day 1	124.5	85.3	85.1	101.2	98.7	95.5	81.4	85.6
Day 2	153.7	121	106.7	123.7	78.4	84.8	80.5	74.5
Day 3	164.6	138.9	122	136.3	93.5	98.4	95.6	87.7

Table 6. Subject Biodex Isokinetic Dynamometer Muscle Strength Indices (continued)

Subject #, (Trial)	Extension 60deg				Flexion 60deg			
ED424(1)	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque
Day 1	142.6	90.7	102.9	128.4	78.3	62	49.1	71.3
Day 2	128.2	93.5	94.6	108.5	74.8	59.9	63.1	70.2
Day 3	124.3	96.3	96.8	108.8	58.8	46.1	48.2	54.3
ED424(2)								
Day 1	109.4	84.8	78.4	89.9	71.9	61.1	52.9	71.2
Day 2	137.8	101.9	112.1	118.8	76.3	60.1	68.6	71.7
Day 3	154.6	116.9	116.1	129.6	75.4	67.1	70.1	69.1
ED424(3)								
Day 1	113.6	89.7	94.8	100.2	84.3	68	75.3	79.9
Day 2	142	113.2	111.3	124.1	74.3	64.7	65.2	70
Day 3	108.8	87.2	74.5	92.3	64.7	53.8	50.8	59.7
ED425(1)								
Day 1	73.1	60	51.7	64	63.3	66.7	49.5	58.6
Day 2	109.4	103.9	75.8	97.7	52.3	53.9	39.5	46.7
Day 3	120.2	112.6	83.3	108.4	43.8	42.3	31.5	39.1
ED425(2)								
Day 1	122.3	110	88.2	118.8	56.5	62.4	47.3	52.4
Day 2	117.9	102.1	79.9	105.7	47.9	45.7	37.5	42.4
Day 3	128.6	111.8	84.4	114	53	52.1	42	51.6
ED425(3)								
Day 1	119	107.6	85	114.3	70.3	72.4	58.5	62.5
Day 2	126.6	109	86	111.2	62.9	62	53.8	59.4
Day 3	122.5	104.3	82.1	103.7	65.1	61.6	46.9	54.2
ED424(1)								
Day 1	160.2	121.8	96.4	134.2	76.7	94.2	69.3	70.3
Day 2	139.1	107.9	93.5	120.1	66.8	76.9	58.7	62.4
Day 3	130.8	112.8	89	116.7	60	71.2	57.9	56.6
ED426(2)								
Day 1	125.8	98.8	83	114.7	68.5	78.4	61.4	58.4
Day 2	123.7	114.3	97.4	114.6	62.7	70.8	59.9	59.7
Day 3	129.6	124.7	102.3	124.4	65.5	86.5	65.3	60.3
ED426(3)								
Day 1	136.7	130.1	101.6	127.1	68	97.9	72.2	65.7
Day 2	139.9	132.1	96.1	122.4	71.4	99.4	65.3	59.3
Day 3	144.6	135.5	16.7	135.9	77.3	102	72.7	69.2

Table 6. Subject Biodex Isokinetic Dynamometer Muscle Strength Indices (continued)

Subject #, (Trial)	Extension 60deg				Flexion 60deg			
ED427(1)	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque	Peak Torque	Max Rep Tot Work	Avg Power	Avg Peak Torque
Day 1	148.5	124.7	102.7	132.5	73.5	77	56.1	60.2
Day 2	164.2	132.9	98.1	130.3	99.5	110.8	74.5	77.4
Day 3	190.1	157.7	124.9	160.3	103	112.9	94.1	94.4
ED427(2)								
Day 1	160.6	147.8	120.4	154.6	90.7	96.1	83.3	85.2
Day 2	174.9	141.5	121.2	156.7	104.9	109.8	82.5	86.4
Day 3	174.6	160.6	130.4	163.1	103.2	121.7	105.4	97.7
ED427(3)								
Day 1	136.4	130.3	104	127.2	93.1	95.2	77.2	82.7
Day 2	166.1	136.2	120	150.5	89.4	92.4	79.3	82.3
Day 3	187	180.1	146.6	146.7	162.6	263.9	104.2	109.8
ED428(1)								
Day 1	132.7	132.4	104.8	126.1	107.6	130.1	90.1	92.4
Day 2	142.2	138.9	115.5	137.7	90.5	107.6	84.6	85.2
Day 3	148.4	135.4	112.3	130.2	68.2	71.6	63.9	62
ED428(2)								
Day 1	130	132.3	98.5	114.4	77.6	94	73.9	70.3
Day 2	137	129	101.1	118.1	81.7	99.6	76.7	74.2
Day 3	165.5	163	123.2	146.5	89.9	115.9	89.7	86.1
ED428(3)								
Day 1	144.1	127.4	95.2	118	69.7	83	63	63.6
Day 2	156.6	135.4	105.4	129.3	87	104.5	71.3	72.4
Day 3	150.8	135.3	97.2	126.5	81.3	105.9	78	73.3
ED429(1)								
Day 1	114.8	105.9	80.6	101.3	76.7	86.4	68.3	74.2
Day 2	103.1	85	79.9	95.8	69.9	66.8	61.2	68.4
Day 3	101.2	90.6	76.3	91.4	60.6	63.1	50.9	56.6
ED429(2)								
Day 1	107.9	88.4	86	100.2	71.8	60	62.6	68
Day 2	112.1	100.5	95.2	105.1	74.6	67.7	56.5	67.3
Day 3	115.9	112.7	95.6	110.6	73.9	84.1	68.1	71.2
ED429(3)								
Day 1	117.4	94.7	89.3	112.3	72.7	75.7	68.2	68.7
Day 2	123.6	104.8	96.5	113.6	81	77.8	73.2	76
Day 3	123.5	105.2	101.2	113.1	75.2	76.7	73	71.6

APPENDIX E: ADDITIONAL TABLES AND FIGURES

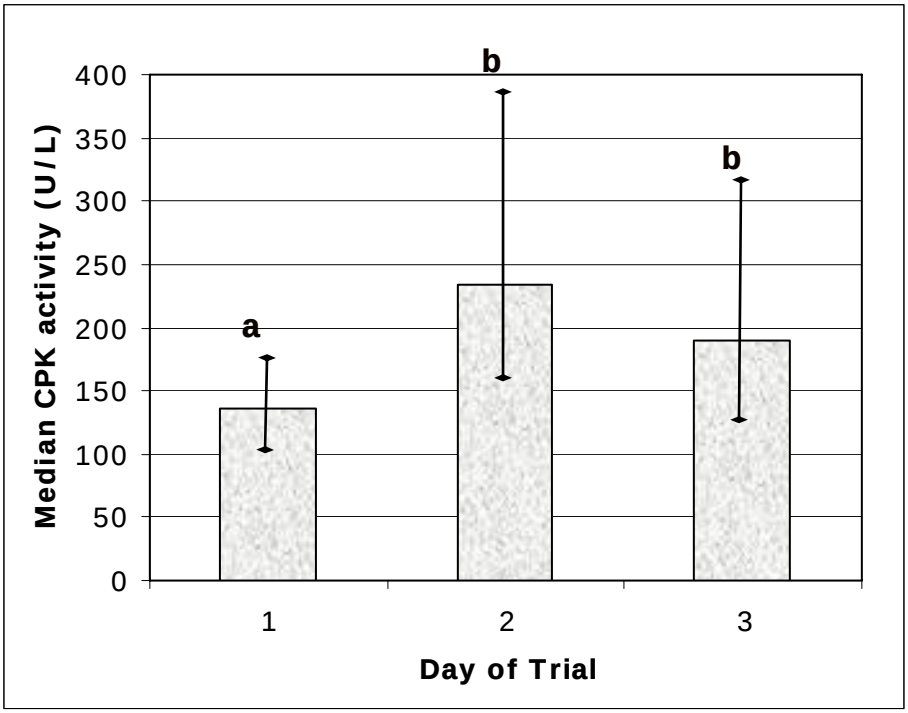
Table 1. Descriptive Statistics of Subjects at Baseline: Level of Exertion (0-10 cm scale) and Physical Activity According to Treatment

Potential Confounding Factors	Treatment Group			Comparison Among Groups[1]
	Placebo	Protein	Carbohydrate	
	Mean ± SD	Mean ± SD	Mean ± SD	p-value
Perceived Level of Exertion (0-10 cm):				
First eccentric set of contractions	8.2 ± 1.6	8.0 ± 1.4	7.8 ± 1.5	0.68
Last eccentric set of contractions	8.6 ± 0.9	8.8 ± 0.6	8.7 ± 0.8	0.66
Recorded Physical Activity (min/day)[2]:				
Rest	1318 ± 102	1334 ± 96	1293 ± 138	0.52
Light	96 ± 99	78 ± 79	104 ± 90	0.62
Moderate	24 ± 39	23 ± 47	40 ± 80	0.57
Heavy	2 ± 7	5 ± 11	2 ± 7	0.40

[1]ANOVA indicated that there were no significant differences among treatment groups.

[2] Physical activity based on 24-hour records

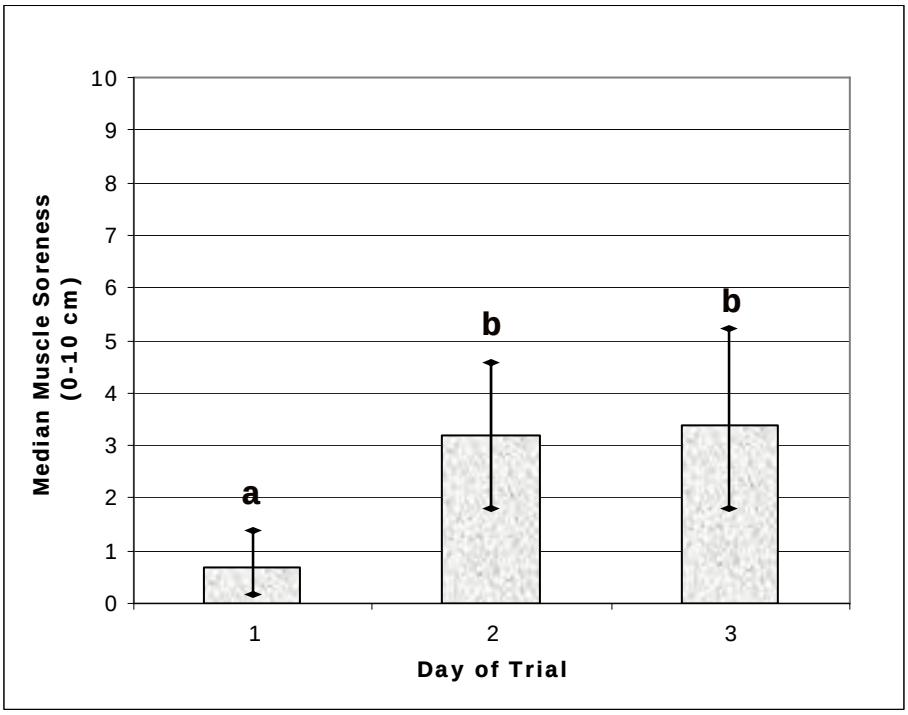
Figure 1. Effect of Day on CPK Activity (U/L) During All Trials Combined



Day:	1	2	3
Maximum:	479	1172	2241
Minimum:	40	86	57

Median values are reported since the data were not normally distributed. Different letters indicate a significant ($p \leq 0.0001$) difference from day to day.

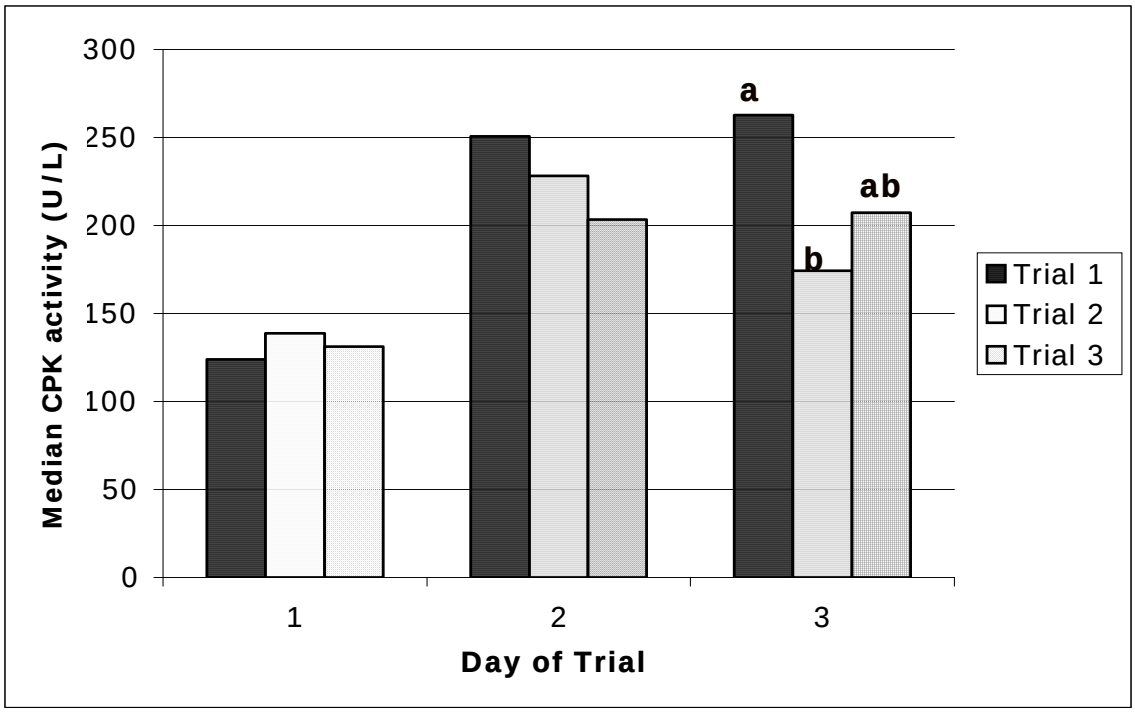
Figure 2. Effect of Day on Perceived Muscle Soreness (0-10 cm) During All Trials Combined



Day:	1	2	3
Maximum:	4.6	9.3	9.9
Minimum:	0.0	0.8	0.0

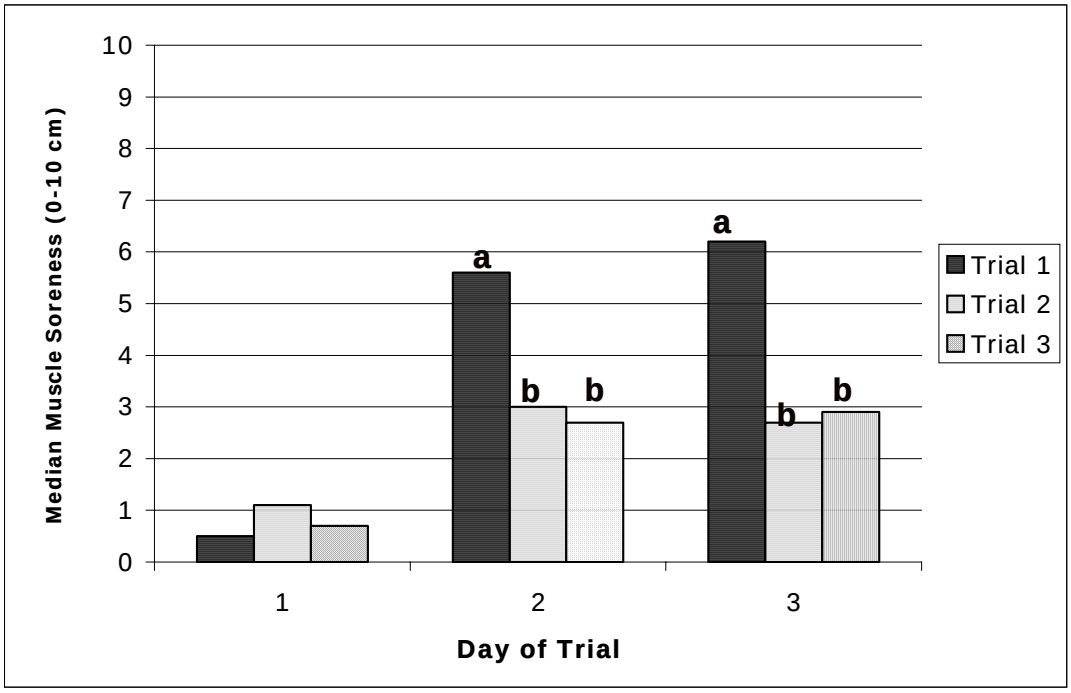
Median values are reported since the data were not normally distributed. Different letters indicate a significant ($p \leq 0.0001$) difference from day to day.

Figure 3. Serum CPK Activity (U/L) According to Trial



Different letters indicate significant (p=0.039) difference from trial to trial.

Figure 4. Perceived Muscle Soreness (0-10 cm) According to Trial



Different letters indicate significant ($p \leq 0.0002$) difference from trial to trial.

Table 2. Descriptive Statistics of Subjects: CPK (U/L) and Muscle Soreness (0-10 scale) at Each Time Point, According to Treatment

	Median			Minima			Maxima		
	Placebo	**PRO**	**CHO**	**Placebo**	**PRO**	**CHO**	**Placebo**	**PRO**	**CHO**
CPK (0 hr)	150	138	131	40	46	53	479	463	290
CPK (0.5 hr)	137	143	128	42	48	40	470	478	290
CPK (24 hr)	251	229	228	103	86	111	924	836	1172
CPK (48 hr)	214	160	194	92	57	93	2241	572	844
Muscle Soreness (0 hr)	0.4	1.0	1.1	0.0	0.0	0.0	4.6	2.3	2.7
Muscle Soreness (0.5 hr)	6.0	5.3	5.9	0.5	0.3	0.7	10.0	9.0	8.6
Muscle Soreness (24 hr)	3.9	2.9	3.1	1.2	0.9	0.8	8.0	7.0	9.3
Muscle Soreness (24.5 hr)	5.5	4.0	4.5	1.1	0.4	0.7	8.7	8.6	9.3
Muscle Soreness (48 hr)	4.4	3.0	3.4	1.0	0.0	1.7	8.7	9.9	8.7
Muscle Soreness (48.5 hr)	4.9	4.8	4.1	1.0	0.5	1.6	9.3	9.6	7.6

Includes medians, minimums, and maximums. Median (minima, maxima) values are reported because CPK and muscle soreness data were not normally distributed.

Table 3. Effect[1] of Day, Trial, and Treatment for Muscle Soreness Measures

	Day Overall	Day 1 *versus* 2	Day 1 *versus* 3	Day 2 *versus* 3	Trial Overall	Trial 1 *versus* 2	Trial 1 *versus* 3	Trial 2 *versus* 3	Treatment Overall	Treatment A *versus* B	Treatment A *versus* C	Treatment B *versus* C
Extension Peak Torque (ft-lbs)	0.018	0.65	0.008	0.028	0.001	0.30	0.0004	0.015	0.25	–	–	–
Extension Max Rep Total Work (ft-lbs)	0.018	0.43	0.047	0.006	0.017	0.20	0.0043	0.14	0.31	–	–	–
Extension Average Power (Watts)	0.75	–	–	–	0.17	–	–	–	0.019	0.31	0.081	0.005
Extension Average Peak Torque (ft-lbs)	0.031	0.93	0.020	0.025	0.003	0.085	0.0007	0.11	0.070	–	–	–
Flexion Peak Torque (ft-lbs)	0.031	0.014	0.039	0.70	<0.0001	0.020	<0.0001	0.018	0.56	–	–	–
Flexion Max Rep Total Work (ft-lbs)	0.034	0.010	0.24	0.15	0.009	0.043	0.003	0.37	0.54	–	–	–
Flexion Average Power (Watts)	0.12	–	–	–	<0.0001	<0.0001	<0.0001	0.45	0.95	–	–	–
Flexion Average Peak Torque (ft-lbs)	0.007	0.003	0.012	0.66	<0.0001	0.008	<0.0001	0.024	0.29	–	–	–

[1] ANOVA for overall effect of day, trial, and treatment

"–" indicated that the follow-up comparisons were not applicable

Treatments: A=placebo, B=protein, C=carbohydrate