THE EFFECT OF MILK CONSUMPTION IMMEDIATELY FOLLOWING RESISTANCE EXERCISE ON PROTEIN DEGRADATION IN UNTRAINED MALES BEFORE AND AFTER A 10-WEEK RESISTANCE TRAINING PROTOCOL

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The effect of milk consumption immediately following resistance exercise on protein degradation in untrained males before and after a 10-week resistance training protocol

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(ABSTRACT)

This study determined the effect of milk or carbohydrate-electrolyte supplementation immediately after resistance exercise on muscle protein breakdown before and after a 10-week resistance training program. Nineteen untrained males, 18-25 years of age, consumed either a carbohydrate-electrolyte (CHO) or milk (MILK) beverage immediately after a strenuous leg resistance exercise bout, both before and after training. Muscle protein breakdown, as estimated by 3-methylhistidine-to-creatinine ratio, was significantly reduced after resistance exercise for both groups, as the ratio was decreased by 19.9% from baseline on the day of resistance exercise. A trend was present for a training effect for 3-methylhistidine-to-creatinine ratio ($p<0.07$), as the reduction from before to after resistance exercise was greater after training. There was no difference in muscle protein breakdown between the groups. One hour after exercise, serum concentrations of amino acids were significantly elevated for MILK and significantly reduced for CHO. Serum glucose was significantly higher for both groups 30 minutes post-exercise than baseline, and serum insulin was greater than baseline 30 minutes and 1 hour after exercise. Serum insulin was significantly greater for CHO than MILK 1 hour after resistance exercise. No effect of training was observed for the response of serum amino acids, glucose, or insulin to resistance exercise with beverage ingestion. In conclusion, although the type of beverage ingested post-exercise affected serum insulin and amino acid concentrations, it did not influence the reduction in muscle protein breakdown observed after resistance exercise. A trend was present for a greater reduction in protein breakdown after training.

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CHAPTER 1: INTRODUCTION

Introduction

Athletes in many sports and physically active individuals from various backgrounds utilize resistance training to increase muscle size and strength. In order to increase muscle size, muscle protein balance must be positive; in other words, muscle protein synthesis must be greater than muscle protein breakdown. Resistance exercise increases both muscle protein synthesis and muscle protein breakdown, and elevation of muscle protein breakdown is actually greater than for synthesis in the fasted state. Therefore, dietary intake may play a very important role in creating a positive protein balance after resistance exercise.

Supplementation after resistance exercise may help to decrease protein breakdown, thereby causing a more positive protein balance. Many resistance trainers take part in post-exercise consumption of one or more of the numerous expensive supplements sold in fitness centers, nutrition stores, and bodybuilding magazines. While research on the effect on muscle protein balance of most of these supplements is lacking, studies involving consumption of supplements simply consisting of amino acids or a mixture of carbohydrate and amino acids have been promising. Researchers have reported that amino acid and carbohydrate-amino acid supplements have caused a decrease in protein breakdown when consumed after resistance exercise. This results in a more positive muscle protein balance, thus enhancing the anabolic effects of resistance training. No research has been done to determine if this acute decrease in muscle protein breakdown after resistance exercise when amino acid or carbohydrate-amino acid supplements are consumed changes as a result of resistance training.

Researchers indicate that supplementation of essential amino acids and carbohydrate after resistance exercise may be effective in acute reduction of protein breakdown. Milk should be considered as a possible supplement, since it contains both carbohydrate and high quality protein. Milk is also naturally occurring and inexpensive, making it an attractive alternative to unproven, expensive supplements.

Statement of the Problem

Athletes and active individuals need proper nutrition to maximize the anabolic effects of resistance training. Supplementation immediately after resistance exercise may be beneficial for causing a positive muscle protein balance by decreasing protein breakdown. Some researchers have discovered that supplementation with carbohydrate, amino acids or a mixture of carbohydrate and amino acids has resulted in a reduction of protein breakdown after resistance exercise. Carbohydrate and amino acid-carbohydrate supplements have been shown to decrease protein breakdown after resistance exercise in separate studies. However, very few researchers have compared the specific effects of these supplements on protein breakdown. Comparison of supplementation with carbohydrate versus a protein-carbohydrate mixture after resistance exercise needs to be done to determine which supplement is most effective for reduction of protein breakdown.

Many researchers have studied the acute changes in protein breakdown with supplementation after a single resistance exercise bout. Research has not been done to determine the effect of resistance training status on acute changes in protein breakdown with supplementation. Protein breakdown has been shown to be lower after resistance exercise for trained individuals. Studies need to be done to determine if training status alters the effect of supplementation on protein breakdown after a resistance exercise bout.

Supplements consumed by resistance trainers are often very expensive and highly processed. Milk may be a better beverage for supplementation since it is natural and relatively inexpensive. Milk also fits the description for supplements that have been shown to reduce protein breakdown after resistance exercise; it contains essential amino acids and carbohydrate. Research is needed to determine the specific effect of milk consumption on protein breakdown after resistance exercise.

Objectives

- To determine the acute effect of post-exercise consumption of a milk or carbohydrate-electrolyte beverage on myofibrillar protein breakdown as estimated with 3-

methylhistidine excretion and hormones related to protein metabolism after a resistance exercise bout.

- To examine the effect of 10 weeks of resistance training on acute myofibrillar protein breakdown after resistance exercise with post-exercise ingestion of a carbohydrate-electrolyte or milk beverage.

Hypotheses

Ho: There will be no difference in muscle protein breakdown or hormones related to protein metabolism during recovery from a resistance exercise bout whether a carbohydrate-electrolyte or milk beverage is consumed after exercise.

Ho: Resistance training will have no effect on the protein breakdown response following a resistance training bout with consumption of a carbohydrate-electrolyte or milk beverage immediately afterwards.

Delimitations

- The subjects were untrained, healthy males between the ages of 18-25 years.
- Subjects had not done any resistance training for at least 3 months prior to the study.
- Subjects were not lactose intolerant.
- Subjects had no injuries or physical contraindications to resistance training.
- Subjects were matched into groups based on weight and dietary calcium intake (determined from dairy frequency form).
- Resistance for exercises in the acute tests was 80% of the subjects 1 RM.
- Subjects' 1 RM was determined at least 2 days before, but no more than 1 week before, the acute tests.
- Personal trainers were present and verified all resistance exercise workouts.
- Subjects were observed consuming the post-exercise supplement beverages.
- All muscle samples were obtained from the vastus lateralis muscle.
- The independent variable for the carbohydrate-electrolyte group was supplementation with Gatorade (5 kcal/kg body weight) immediately after resistance exercise in the

acute tests and immediately after each resistance training bout. The independent variable for the milk group was supplementation with low fat chocolate milk (5 kcal/kg body weight) immediately after resistance exercise in the acute tests and immediately after each resistance training bout.

- The dependent measures were body weight, strength gains, serum leucine, serum total amino acids, serum essential amino acids, serum glucose, serum insulin, serum IGF-1, urinary 3-methylhistidine, and urinary creatinine.

Limitations

- Subjects were free living. Thus, self-reported dietary intake cannot be verified.
- Results can only be applied to individuals of similar gender, age, and training status.
- There was no control group in this study to determine the effect of resistance exercise on protein breakdown without supplementation.
- The energy content of the meat-free diet was 30 kcal/kg body weight, which may not have accurately represented the energy requirement of the subject.
- There was no control on timing of food consumed on the diet control days. Thus, some subjects may have had a longer fast than others before the acute test.
- The sensitivity of 3-methylhisitidine excretion as a marker for protein breakdown may not have been high enough to detect a change in muscle protein breakdown of the leg muscles.
- 3-Methylhistidine excretion may not have been measured long enough to accurately assess the effects on muscle protein breakdown.
- Several subjects did not provide complete 24-hour collections of their urine.
- Several urine sample bottles were overfilled, compromising the integrity of these samples.
- Several subjects were unable to complete all exercises at 80% 1 RM in the acute test, resulting possibly from fatigue, awkwardness of the strength equipment (seat on leg press could not be adjusted to accommodate subjects' heights), or pain from the biopsies.

Definitions and Symbols

- **Isotope infusion** — Infusion of a labeled isotope of an amino acid into the blood (usually phenylalanine) to determine the isotope's uptake and decay in the muscle; used as measures of muscle protein synthesis and breakdown.
- **3-Methylhistidine** — A product of degradation of contractile proteins that is excreted by the kidneys without reutilization.
- **myofibrilla** — One of many contractile filaments that make up a striated muscle fiber.
- **RM** — Repetitions Maximum, amount of weight for an exercise that can be lifted a certain amount of times.
- **Concentric** — Action that occurs when a muscle shortens.
- **Eccentric** — Action that occurs when a muscle lengthens.
- **Essential Amino Acids** — The nine amino acids that can not be made in the human body, and therefore are essential in the diet.
- **IGF-1** — Insulin-like growth factor-1
- **IGFBP** — Insulin-like growth factor-1 binding proteins
- **ANOVA** — Analysis of Variance
- **Carbohydrate-Electrolyte Group (CHO)** — The group of subjects (n=9) given 5 kcal/kg body weight of Gatorade immediately after resistance exercise in the acute tests and immediately after each resistance training bout.
- **Milk Group (MILK)** — The group of subjects (n=10) given 5 kcal/kg body weight of low fat chocolate milk immediately after resistance exercise in the acute tests and immediately after each resistance training bout.

Basic Assumptions

- Subjects had not done any resistance training for at least 3 months prior to the study.
- Subjects' calcium intake on the days when the food record was kept was representative of their normal intake.
- Subjects abstained from nutritional supplements during the resistance training period.
- Subjects abstained from meat for 3 days before the acute tests and on the day of the acute tests.
- Subjects followed the food checklist, designed to provide them with 30 kcal/kg body weight, given to them for the 3 days before the acute tests and the day of the acute tests.
- Subjects fasted for 12 hours before the acute tests.
- Subjects collected all of their urine for the day before the acute tests and the day of the acute tests.
- Muscle biopsies did not affect performance of the resistance exercises completed in the acute tests and during the training program.

CHAPTER 2: LITERATURE REVIEW

Introduction

The effect of resistance exercise on muscle protein breakdown has been studied in numerous experiments. Various researchers have reported a significant increase in muscle protein breakdown after a strenuous resistance exercise bout (5, 23, 37, 46, 47). This elevation becomes greater as intensity of resistance exercise is increased (5, 38). Although most researchers have indicated an increase in protein breakdown after resistance exercise, results from some studies have been conflicting, noting that no change (24, 25) or a decrease (36) in protein breakdown was present after resistance exercise.

The method utilized to measure muscle protein breakdown after resistance exercise may affect the results that are found. Isotope infusion has consistently shown an increase in protein breakdown after resistance exercise. Researchers indicate that urinary 3-methylhistidine is also a valid marker for myofibrillar protein degradation, since the amount of nonskeletal muscle protein degradation can be assumed to be constant for all subjects during resistance exercise (16). Despite this finding, researchers using urinary 3-methylhistidine as a measure of protein breakdown have produced conflicting results. The sensitivity of urinary 3-methylhistidine as a measure of protein breakdown is not completely known, and measurements may need to be done for days after resistance exercise to determine the effect on protein breakdown.

Researchers found that supplementation with amino acids or carbohydrate with amino acids may decrease the protein degradation experienced after resistance training (6, 40). On the other hand, in a study by Roy et al. (42), supplementation of carbohydrate or carbohydrate with protein and fat had no effect on protein breakdown after resistance exercise. Further research is needed to determine the specific effects of supplementation on protein breakdown after resistance exercise. Milk beverages could be beneficial supplements to decrease protein degradation, since milk is a mixture of carbohydrate, very high quality protein, and fat. Supplementation with carbohydrate and amino acids may reduce protein breakdown by increasing plasma amino acids, insulin, and insulin-like growth factor-I (IGF-1).

Muscle Protein Breakdown and Resistance Exercise

Research from various studies indicates that there is an increase in muscle protein degradation after resistance exercise (5, 23, 38, 46, 47). In a study by Biolo et al. (5), subjects performed a strenuous lower body resistance training bout, including 5 sets of 10 repetitions at 12 RM for incline leg press, and 4 sets of 8 repetitions at 10 RM for squats, leg curls, and leg extensions (5). Isotope infusion revealed protein degradation, by determining the release of phenylalanine from muscle, to be 51% higher than resting levels (5).

Researchers have reported an increase in muscle protein breakdown for up to 48 hours after resistance exercise (37, 46). Phillips et al. (37) measured muscle protein breakdown after resistance exercise using isotope infusion. Subjects performed 8 sets of 8 repetitions of knee extensor exercise, concentric or eccentric, at 80% of their 1 RM. Muscle protein breakdown was increased by 31% at 3 hours post-exercise, and by 18% at 24 hours post-exercise (37). Protein breakdown returned to normal levels by 48 hours after exercise (37).

Researchers have shown that a significantly lower amount of muscle protein breakdown is present after resistance exercise for trained subjects when compared with those who are untrained (38, 46). In a study by Phillips et al. (38), muscle protein breakdown after a resistance exercise bout was unchanged for trained subjects, but catabolism was increased by 37 +/- 5% for subjects who had not taken part in prior resistance training.

Resistance exercise resulted in an increase in 3-methylhistidine excretion, a marker for protein breakdown, in studies by Hickson et al. (23) and Viru and Seli (47). Viru and Seli (47) found an increase in 3-methylhistidine excretion for 24 hours after resistance exercise, with the greatest increase occurring between 12-24 hours after resistance exercise. Subjects took part in a resistance training program for 8 weeks, and were divided into high intensity resistance training (exercises at 70% of 1 RM), and low intensity resistance training (exercises at 50% of 1 RM) groups. Urinary 3-methylhistidine was increased for all subjects for the first 3 weeks (47). Urinary 3-methylhistidine then returned to normal levels in the subjects performing low intensity

resistance exercise, but excretion remained elevated for 6 weeks in the subjects performing high intensity resistance training (47). This shows that muscle protein breakdown increases when resistance training intensity is higher. Although 70% 1 RM was designated as high intensity group in this study, this intensity is not considered to be high intensity resistance exercise (27).

While most researchers have determined that resistance exercise increases protein degradation, there have been studies where the measures of protein degradation were not increased after resistance exercise (24, 25, 36). In a study by Paul et al. (36), 3-methylhistidine-to-creatinine ratio decreased at 24 hours and 48 hours after exercise when compared to before exercise. Other experimenters revealed no change in 3-methylhistidine excretion after resistance exercise (24, 25).

Protein breakdown may not be of a significant magnitude to affect 3-methylhistidine excretion after a single bout of resistance exercise, thus repeated bouts may be needed to see a substantial effect. In a study done by Pivarnik et al. (39), subjects alternated upper body or lower body resistance exercise for 11 days. Urinary 3-methylhistidine excretion was increased after the third day of resistance exercise and remained elevated above resting levels during the rest of the resistance training period (39). Excretion of 3-methylhistidine increased on the third day of weight training, but not on the first 2 days (39).

Results from research done to determine the effect of resistance exercise on protein breakdown appear to be affected greatly by both the method utilized and the intensity of the resistance bout. Intensity of the resistance bout must be strenuous to result in an increase in muscle protein breakdown. Research using isotope infusion has been the most consistent and is likely more sensitive to show an increase in protein breakdown with resistance exercise. Researchers using 3-methylhistidine excretion to determine muscle protein breakdown have shown mixed results. Some researchers found elevation of protein breakdown after resistance exercise, while others reported no change or a decrease in protein breakdown. Further research needs to be done to determine the sensitivity of 3-methylhistidine to measure protein breakdown. Yarasheski et al. (50) found an increase in protein breakdown after resistance exercise by using isotope

infusion, but there was no effect on 3-methylhistidine excretion. As a result, the sensitivity of 3-methylhistidine as a marker for protein breakdown may be less than isotope infusion. Despite some inconsistencies in research, most researchers have shown an increase in muscle protein degradation as a result of a strenuous resistance exercise bout.

3-Methylhistidine as a Marker for Skeletal Muscle Protein Breakdown

The results of various studies indicate that 3-methylhistidine excretion is an accurate marker for skeletal muscle protein degradation. Since animal meat contains 3-methylhistidine, subjects may not consume meat prior to measurement of 3-methylhistidine excretion. Excretion must be measured after 2 days of a meat-free diet, since 3-methylhistidine in the body is completely endogenous after this amount of time (31). When diet is controlled, researchers determined that 3-methylhistidine excretion was an effective measure of muscle protein degradation (16).

Not all researchers support the use of 3-methylhistidine excretion to measure muscle protein catabolism. A portion of the 3-methylhistidine excreted from the body is from nonskeletal muscle (39, 41). Researchers are in disagreement about the significance of the nonskeletal sources of 3-methylhistidine to excretion. Long et al. (30) stated that the contribution of nonskeletal sources to 3-methylhistidine excretion appears to be negligible. While the researchers that support the use of 3-methylhistidine claim that the contribution of these nonskeletal sources is very small, some researchers believe that excretion by these sources makes 3-methylhistidine excretion an inaccurate measure for skeletal protein breakdown (41). Rennie and Millward (41) mentioned that the nonskeletal sources of 3-methylhistidine turn over at a much faster rate than skeletal muscle. As a result, some experimenters believe that 3-methylhistidine excretion is an invalid marker for protein catabolism. Researchers have taken this issue into consideration, claiming that excretion from nonskeletal sources can be estimated accurately (41).

In the study previously mentioned by Yarasheski et al. (50), there was an increase in protein breakdown after resistance exercise by using isotope infusion, but there was no

effect on 3-methylhistidine excretion. As a result, the sensitivity of 3-methylhistidine as a marker for protein breakdown may be less than isotope infusion. As shown by Pivarnik et al. (39), 3-methylhisitidine may have to be measured for a longer period of time to determine the effect of resistance exercise on protein breakdown. While there are some questions about the use of 3-methylhistidine excretion to estimate muscle protein breakdown, most researchers agree that it is a valid procedure. The test is easy to administer, and it is noninvasive to the subject.

Amino Acid Supplementation After Resistance Exercise and Muscle Protein Degradation

According to research, infusion of amino acids after resistance exercise may cause a decrease in muscle protein degradation (6). In the study by Biolo et al. (6), 6 untrained males were given an infusion of amino acids at rest and after resistance exercise (in separate trials at least 1 week apart). Infusion of amino acids after resistance training decreased muscle protein breakdown to resting levels. Findings from this study are beneficial for determining the effects of amino acid supplementation after resistance exercise, but they cannot be applied practically. While infusion is not done often, oral consumption of drinks consisting of amino acids after resistance exercise is common. Therefore, determining the effect of oral consumption of amino acids on protein breakdown after resistance exercise has greater practical significance.

Some researchers have reported an enhancement of muscle protein balance with oral supplementation of specific amino acids after resistance exercise. Tipton et al. (44) studied the potential value of amino acid consumption after resistance exercise. Their research group discovered that a supplement with only essential amino acids was equally effective in improving muscle protein balance after resistance exercise as a mixed amino acid supplement. Six subjects (3 males and 3 females) completed an intense leg-resistance exercise bout, and consumed a solution containing 40 g of mixed amino acids, a solution containing 40 g of essential amino acids, or a placebo afterwards (100 mL at a time, starting immediately and continued at 20 minute intervals). Muscle protein balance was similar in the mixed amino acid (17 ± 13 nmol/min · 100 mL leg volume) and essential amino acid (29 ± 14 nmol/min · 100 mL leg volume) trials. Muscle protein

balance was much more negative in the placebo trial (-50 ± 23 nmol/min · 100 mL leg volume) than in the amino acid trials. As a result, Tipton et al. (44) concluded that consumption of amino acids after resistance exercise improves protein balance and that nonessential amino acids are not necessary to improve muscle protein balance after resistance exercise.

The improvement of muscle protein balance in the study by Tipton et al. (44) was a result of increased muscle protein synthesis; there were no differences in protein breakdown, as measured by isotope infusion, after resistance exercise from resting with placebo or amino acid supplementation. A more strenuous resistance exercise bout may have been needed to stimulate protein breakdown for the placebo.

Tipton et al. (44) also compared muscle amino nitrogen uptake via oral ingestion in this study with muscle amino nitrogen uptake after amino acid infusion from a previous study done by the same researchers (6). Researchers found that 32% of the amino nitrogen and 34% of the infused amino nitrogen was taken up by muscle. This shows that oral ingestion of amino acids and infusion of amino acids are equally effective in promoting muscle amino acid uptake. As a result, infusion and oral supplementation may cause the same relative decrease in protein breakdown after resistance exercise.

Carbohydrate and Carbohydrate-Protein Supplementation After Resistance Exercise and Protein Degradation

One research group showed that carbohydrate ingestion after resistance exercise also affects muscle protein degradation (43). Roy et al. (43) concluded that ingestion of 1g/kg body weight of carbohydrate immediately after and 1 hour after resistance training caused a decrease in muscle protein degradation by measuring a lower amount of urinary 3-methylhistidine. In this study, 8 healthy males did 4 sets of 8-10 repetitions for leg press and knee extension at 85% of 1 RM. A glucose drink or Nutrasweet placebo was given afterwards. Urinary 3-methylhistidine excretion was significantly lower when the subjects were given the glucose supplement (mean 110 μg/mol for glucose vs. 120.14 μg/mol for placebo) (43). Therefore, carbohydrate ingestion was interpreted to cause a decrease in muscle protein degradation. A limitation to this study is that there were no

pre-exercise 3-methylhistidine excretion values to compare to the post-exercise values. Thus, it cannot be determined whether exercise influenced protein breakdown.

A study by Rasmussen et al. (40) was done to determine the effects of timing of an essential amino acid-carbohydrate supplement on protein metabolism after resistance exercise. Subjects performed 10 sets of 8 repetitions for leg press and 8 sets of 8 repetitions for leg extension. Both exercises were done at 80% of the subjects' 1 RM. The essential amino acid-carbohydrate supplement or a placebo was given at 1 hour or 3 hours after exercise. Protein breakdown, determined by isotope infusion, was not significantly changed from resting levels when the oral amino acid-carbohydrate supplement was ingested at 1 hour and 3 hours after exercise (40). Since protein breakdown is usually increased after resistance exercise when fasting, the supplement may have been successful in significantly lowering protein breakdown (40).

A limitation to the study by Rasmussen et al. (40) is that the effect of immediate consumption of the supplement on protein breakdown was not studied. The experiment was done to determine if time of consumption of the supplement affected protein breakdown. Therefore, it would be most logical to study the effect of supplementation immediately after resistance exercise, closer to the time when protein breakdown is initially elevated, on reduction of protein breakdown. Another limitation to this study was that there was not a control group. Thus, it is not known if the amino acid-carbohydrate supplement caused a reduction in protein breakdown.

Similar effects of a post-exercise nutrition supplement on protein breakdown resulted in a study by Tipton et al. (45). In this study, an essential amino acid-carbohydrate supplement was given either immediately before or immediately after a resistance training bout. Subjects performed 10 sets of 8 repetitions for leg press and 8 sets of 8 repetitions of leg extension, both at 80% of subjects' 1 RM. Protein breakdown, as determined by isotope infusion, was not changed after exercise from resting levels when the essential amino acid-carbohydrate supplement was given immediately before or immediately after resistance exercise (45). As resistance exercise typically increases protein breakdown, this is interpreted as an anabolic response to nutritional intervention. A limitation to this study was that there was not a control group.

Thus, it is not known if the amino acid-carbohydrate supplement caused a reduction in protein breakdown.

In a study by Roy et al. (42), there was no effect on protein breakdown when subjects were given a carbohydrate or carbohydrate/protein/fat supplement immediately after resistance exercise. Subjects completed 3 sets of 10 repetitions at 80% 1 RM for 8 resistance exercises. The subjects were given a carbohydrate supplement, carbohydrate/protein/fat supplement, or a placebo after exercise. Protein breakdown, determined by measuring urinary 3-methylhistidine, was similar in all 3 treatments, showing no decrease as a result of carbohydrate or carbohydrate/protein/fat supplementation (42).

The study by Roy et al. (42) may have been limited by the method used to measure protein breakdown, measurement of urinary 3-methylhistidine. The findings of this study are questionable since measurement of 3-methylhistidine to estimate protein breakdown has produced less consistent results than isotope infusion. The sensitivity of 3-methylhistidine measurement is not known, and measurement may need to be done for days after resistance exercise to determine the effect of resistance exercise on protein breakdown. Also, the study is limited since there were no pre-exercise 3-methylhistidine excretion values to compare to the post-exercise values, making it impossible to determine whether exercise influenced protein breakdown.

One research group indicated a possible effect of ingestion of milk, a natural food containing carbohydrate and high quality protein, on muscle damage. Cade et al. (10) gave subjects a sucrose drink or a milk protein supplement after exercise. By measuring creatine kinase, Cade et al. (10) discovered that the milk protein supplement decreased muscle damage after an intense swim workout. Cade et al. (10) emphasized the value of supplementation of a high quality protein drink after high-intensity exercise for replacement of essential amino acids broken down during exercise. Consumption of milk, which is this type of supplement, after exercise may lead to a more rapid repair of muscle tissue (10). Results from this study cannot be applied specifically to protein breakdown, since muscle damage is not the same as muscle protein breakdown. Research needs to be done to find the specific effect of milk consumption on protein

breakdown after resistance exercise.

Researchers have indicated that the protein in milk is very high in quality. Bos et al. (9) determined the net postprandial protein utilization, an index of protein quality, of milk to be 81%. Measurement by Gaudichon et al. (21) produced similar results, measuring the net postprandial protein utilization of milk to be 80%. This study went a step further and combined sucrose with milk protein. Net postprandial protein utilization was higher for this group (85%), suggesting that sucrose (or possibly carbohydrate in general) may cause greater absorption of protein (9).

Despite a growing number of studies on supplementation after resistance exercise, researchers have yet to determine which nutrients are most beneficial in decreasing protein breakdown. Some researchers believe that amino acids alone are sufficient in causing the greatest decrease in protein degradation, while others claim that carbohydrate should be included along with amino acids. More research needs to be done to find the effects of supplementation with different kinds of amino acid mixtures or carbohydrate-amino acid mixtures on protein breakdown. Results from research have been conflicting, amino acid and carbohydrate-amino acid supplements resulted in a decrease in protein breakdown in some studies, but no change in protein breakdown was present in other studies. Milk appears to be a promising candidate for post-exercise consumption, since it is a naturally occurring beverage that contains carbohydrate, high quality protein, and fat, but more research involving milk consumption and resistance exercise needs to be done.

Insulin and Protein Degradation

Researchers that show a decrease in protein degradation with supplementation suggest hyperinsulinemia as the link with this result (2, 14, 20, 22, 34). Fukagawa et al. (20) administered different amounts of insulin to subjects, and determined that plasma 3-methylhistidine, a marker of protein breakdown, decreased as the insulin dose was increased. In a study by Denne et al. (14), there was a 42% decrease in the release of phenylalanine (marker for muscle protein breakdown) in the leg given the infusion, and a 15% decrease in the release of phenylalanine in the whole body as a result of insulin infusion. Biolo et al. (7) measured muscle protein breakdown after resistance exercise

with or without insulin infusion. Subjects given an infusion of insulin had a significant reduction of protein breakdown after resistance exercise compared to controls (7).

Researchers have shown that infusion of a combination of insulin and amino acids caused a decrease in protein degradation (2, 18). In a study by Bennet et al. (2), subjects given insulin-amino acid infusions had a 29% greater decrease in the release of phenylanine from muscle tissue, a marker for protein degradation, than those given just amino acid infusions. Flakoll et al. (18) used isotope infusion, and found that infusion of insulin decreased protein degradation by 35% when compared to basal concentration. When amino acids were infused along with insulin, protein degradation was decreased by 70% when compared to basal concentration (18).

The mechanisms for decreasing protein degradation appear to be different for insulin than amino acids. Insulin appears to possibly decrease protein degradation by stimulating amino acid transport, while dietary amino acids may be important to increase plasma amino acid concentration (1). Reduction of protein breakdown may be affected by circulation of plasma amino acids (1). Protein supplementation supplies the amino acids, and insulin aids in the transport of these amino acids. The mixture of insulin and amino acids may be beneficial since amino acids have been shown to decrease muscle protein degradation in tissues that are not sensitive to insulin (11).

The decrease in muscle protein breakdown resulting from hyperinsulinemia may be augmented by exercise, since exercise causes an increase in skeletal muscles' sensitivity to insulin (1, 11, 13). This effect also can be increased through continued exercise training. As a person trains on a regular basis, they develop a greater sensitivity to insulin after exercise (13).

Supplementation with carbohydrate after resistance exercise may be effective in decreasing protein breakdown as a result of increasing insulin concentration. In the study mentioned above by Roy et al. (43), insulin concentration was increased when the subjects consumed the carbohydrate supplement. The carbohydrate supplement was shown to decrease protein breakdown, which may be related to elevation of insulin. Insulin concentration was elevated for subjects given amino acid-carbohydrate supplements in the studies involving resistance training done by Rasmussen et al. (40),

Tipton et al. (45), and Kraemer et al. (28). Since both carbohydrate and carbohydrate-amino acid supplements have been shown to increase serum insulin, either nutritional intervention may decrease muscle protein breakdown.

In the study by Biolo et al. (6), infusion of amino acids after resistance exercise decreased protein breakdown, but there was a very small effect on insulin concentration. Therefore, reduction in protein breakdown resulted from a different mechanism, most likely an increase of the plasma amino acid concentration (1), than an elevation of serum insulin concentration. Elevation of insulin, from the addition of carbohydrate, may have caused a greater reduction in protein breakdown, by stimulating amino acid transport (1), than amino acids alone.

Insulin concentration appears to have a significant effect on reduction of protein breakdown. Fukagawa et al. (20) showed that as insulin concentration was increased, protein breakdown was reduced. Addition of amino acids may cause a greater reduction in protein breakdown. A protein-carbohydrate supplement may be beneficial to decrease protein breakdown, since protein can elevate circulating amino acid concentrations and carbohydrate intake will increase insulin concentration (1). Exercise training may also aid in reduction of protein breakdown by improving insulin sensitivity.

IGF-1 and Protein Degradation

IGF-1 has been shown to have significant anabolic effects, causing an increase in lean mass when concentrations are elevated for subjects who had IGF-1 deficiency (32). In a study by Bermon et al. (3), an increase in IGF-1 was present for elderly subjects immediately after (+17.7%) and 6 hours after (+7.5%) resistance exercise. This increase was determined to be acute, as 8 weeks of resistance training had no effect on serum IGF-1 concentration (3).

Some researchers have reported no acute effect from resistance exercise on IGF-1 concentration (26, 33, 35). IGF-1 was elevated during and after a resistance exercise bout when compared to pre-exercise concentration in a study by McCall et al. (33). However, no acute alterations in IGF-1 concentration resulted after correction for changes in plasma volume (33).

Nindl et al. (35) measured IGF-1 concentration for subjects after a heavy resistance bout, and determined that there was no change in IGF-1 compared to resting controls, but changes were found in IGF-1 binding proteins (IGFBPs). At 1 hour after exercise, IGFBP-3 was elevated 7.6% above the controls, while IGFBP-2 was not changed. IGFBP-3 was not different than resting levels the next morning, but IGFBP-2 was elevated 12.2% compared to the control group. Nindl et al. (35) concluded that heavy resistance exercise could lead to alterations in the IGF-1 system. These changes may aid in adaptations associated with increased strength.

Borst et al. (8) measured strength gains and circulating IGF-1 in subjects participating in a resistance training program. Circulating IGF-1 increased 20% above baseline concentrations and strength was significantly increased after 13 weeks of training, but there were no further increases after 25 weeks for strength or IGF-1. Borst et al. (8) concluded that circulating IGF-1 may, in part, mediate increased strength that results from resistance training.

IGF-1 concentration may increase with increasing availability of amino acids in the body (19). In the previously mentioned study by Kraemer et al. (28), pre-exercise IGF-1 concentration was higher on the second and third days of resistance exercise when subjects were given a protein-carbohydrate supplement 2 hours before and immediately after resistance exercise compared to a group that fasted and performed the resistance exercise. Resistance exercise did not result in any acute changes in IGF-1 for either group (28).

Chandler et al. (12) measured IGF-1 before and after resistance exercise when subjects were given water (control) or 1 of 3 supplements: protein, carbohydrate, or protein-carbohydrate. The supplements or water were given immediately after and 2 hours after exercise. IGF-1 concentration was not affected by any of the supplements when compared to the control (12). Resistance exercise did not change IGF-1 from resting concentrations (12).

Research groups have found that elevated IGF-1 concentration caused a decrease in protein breakdown in animals and humans (4, 17, 29). Berneis et al. (4) measured leucine oxidation to estimate protein degradation, and determined that administration of

growth hormone plus IGF-1 resulted in a greater decrease in protein degradation than growth hormone alone for catabolic subjects (2.5% increase in leucine oxidation for group given growth hormone, 17.7% decrease in leucine oxidation for group given growth hormone plus IGF-1). The placebo group had an increase in protein catabolism, the growth hormone group had no change in protein catabolism, and the growth hormone and IGF-1 group had a decrease in protein catabolism (4). Although IGF-1 and insulin appear to affect protein breakdown in similar ways, a study by Laager et al. (29) concluded that IGF-1 caused a greater decrease in protein breakdown than insulin. IGF-1 was shown to use insulin receptors in addition to IGF-1 receptors (29).

Weight training has been shown to possibly result in alterations in IGF-1 concentration, which may in turn affect protein breakdown and subsequent strength gains. Although results from studies have been conflicting, IGF-1 or IGF binding protein concentration may be affected acutely by a resistance exercise bout. The effect of chronic training on IGF-1 concentration has also not been fully determined. Borst et al. (8) determined that IGF-1 concentration was increased as strength was increased with training. Supplementation may affect IGF-1 concentration, which in turn may affect protein breakdown and strength gains. More research is needed to find the specific effects of acute resistance exercise, resistance exercise training, and supplementation on IGF-1 concentration.

CHAPTER 3:

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The effect of milk consumption immediately following resistance exercise on protein degradation in untrained males before and after a 10-week resistance training protocol

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ABSTRACT

This study determined the effect of milk or carbohydrate-electrolyte supplementation immediately after resistance exercise on muscle protein breakdown before and after a 10-week resistance training program. Nineteen untrained males, 18-25 years of age, consumed either a carbohydrate-electrolyte (CHO) or milk (MILK) beverage immediately after a strenuous leg resistance exercise bout, both before and after training. Muscle protein breakdown, as estimated by 3-methylhistidine-to-creatinine ratio, was significantly reduced after resistance exercise for both groups, as the ratio was decreased by 19.9% from baseline on the day of resistance exercise. A trend was present for a training effect for 3-methylhistidine-to-creatinine ratio ($p<0.07$), as the reduction from before to after resistance exercise was greater after training. There was no difference in muscle protein breakdown between the groups. One hour after exercise, serum concentrations of amino acids were significantly elevated for MILK and significantly reduced for CHO. Serum glucose was significantly higher for both groups 30 minutes post-exercise than baseline, and serum insulin was greater than baseline 30 minutes and 1 hour after exercise. Serum insulin was significantly greater for CHO than MILK 1 hour after resistance exercise. No effect of training was observed for the response of serum amino acids, glucose, or insulin to resistance exercise with beverage ingestion. In conclusion, although the type of beverage ingested post-exercise affected serum insulin and amino acid concentrations, it did not influence the reduction in muscle protein breakdown observed after resistance exercise. A trend was present for a greater reduction in protein breakdown after training.

Keywords: muscle protein breakdown, 3-methylhistidine, resistance exercise, insulin

Introduction

Various research groups have shown that muscle protein breakdown was elevated after strenuous resistance exercise (3, 14, 22, 30, 31). This increase may be present for up to 48 hours after resistance exercise (22, 30). Researchers have determined that a significantly lower amount of muscle protein breakdown was present after resistance exercise for trained subjects when compared to those who are untrained (23, 30). In a study by Phillips et al. (23), muscle protein breakdown after a resistance exercise bout was unchanged for trained subjects, but catabolism was increased by 37 +/- 5% for subjects who had not taken part in prior resistance training.

The method implemented to measure muscle protein breakdown after resistance exercise has substantial bearing on the results gathered from the experiment. Isotope infusion is the preferred method of measurement, since researchers using this procedure have consistently reported an increase in muscle protein breakdown after strenuous resistance exercise (3, 22, 30). Measurement of 3-methylhistidine excretion has been a less consistent marker for muscle protein breakdown. While 3-methylhistidine excretion increased after resistance exercise in some studies (14, 31), 3-methylhistidine has remained unchanged (15, 16) or even decreased (21) in other experiments. In a study by Paul et al. (21), 3-methylhistidine-to-creatinine ratio was reduced below baseline at 24 hours and 48 hours after resistance exercise.

Various research studies have been completed in recent years to determine a method to attenuate the elevation in protein breakdown that is present after resistance exercise. Supplementation with protein or carbohydrate after exercise has provided promising results. In a study by Biolo et al. (4), infusion of amino acids after resistance exercise reduced muscle protein breakdown to resting levels, most likely by increasing amino acid availability. Tipton et al. (28) discovered that essential amino acids are as effective as a mixture of all amino acids in enhancement of muscle protein balance after resistance exercise. Therefore, it appears that nonessential amino acids are not required for reduction of protein breakdown after resistance exercise.

Supplementation of carbohydrate after resistance exercise reduced protein

breakdown in an experiment by Roy et al. (27). When a carbohydrate supplement was given immediately and 1 hour after resistance exercise, 3-methylhistidine excretion was decreased significantly when compared to a placebo. The reduction in protein breakdown caused by the carbohydrate supplement may be a result of enhanced amino acid transport from hyperinsulinemia (1).

A beverage containing a combination of carbohydrate and amino acids may be the most effective form of supplementation for reduction of protein breakdown after resistance exercise. Amino acids provided in this mixture induce hyperaminoacidemia to increase amino acid availability, and carbohydrate causes stimulation of amino acid transport through hyperinsulinemia (1). Previous researchers using isotope infusion have reported no change in protein breakdown after resistance exercise when compared to resting when an amino acid-carbohydrate supplement was consumed (25, 29). This is interpreted as a reduction in protein breakdown because protein breakdown has been shown to be increased significantly above resting levels after resistance exercise in the fasted state (3, 14, 22, 30, 31).

Although carbohydrate and amino acid-carbohydrate supplements have resulted in reduction in protein breakdown in separate studies, very little research has been carried out to compare these supplements. One experiment by Roy et al. (26) was done to investigate this, comparing the effect of a carbohydrate supplement to a carbohydrate/protein/fat supplement fed after exercise on muscle protein breakdown after a resistance exercise bout. Although each supplement stimulated protein synthesis, there was no effect on protein breakdown, as estimated by 3-methylhistidine excretion, for either supplement when compared to a placebo. This highlights the inconsistencies found regarding the effects of various dietary supplements on muscle protein breakdown following resistance exercise. Further research comparing beverages with carbohydrate to carbohydrate/protein/fat mixtures is necessary to determine which supplement is more effective for decreasing protein breakdown after resistance exercise.

Milk may be an effective beverage for reduction of muscle protein breakdown when consumed after resistance exercise. Milk is naturally occurring, and is made up of a mixture of carbohydrate, protein, and fat. This macronutrient composition may be

favorable for attenuation of protein breakdown. The protein in milk is high quality, and it consists of many essential amino acids, protein components proven to enhance protein balance after resistance exercise.

Minimal research has been completed to study the effect of milk on muscle protein breakdown after resistance exercise. Cade et al. (6) provided milk for subjects to consume after an intense swim workout. Cade et al. (6) measured creatine kinase, a marker for muscle damage, and discovered that milk reduced muscle damage after swimming. Although muscle damage is not the same as muscle protein breakdown, this research suggests that milk may be beneficial in recovery from strenuous exercise. Wojcik et al. (33) reported that a milk beverage fed after strenuous eccentric exercise tended to reduce serum creatine kinase and 3-methylhistidine excretion compared to a carbohydrate or placebo beverage, but these results were not significantly different. Further research must be done to determine the effect of milk consumption after resistance exercise on muscle protein breakdown.

The purpose of our study was twofold: 1) to determine the effect of consumption of a milk or carbohydrate-electrolyte beverage on muscle protein breakdown after an acute resistance exercise bout, and 2) to examine the effect of 10 weeks of resistance training on the acute response of muscle protein breakdown after resistance exercise with milk or carbohydrate-electrolyte consumption.

Methods

Subjects

Nineteen untrained male subjects between the ages of 18 and 25 years were recruited for this study. Subjects had not participated in resistance training for at least 3 months prior to the start of the study. Subjects gave their informed consent, and the study was approved by the Institutional Review Board. Screening was done to eliminate subjects with lactose intolerance, health problems that would make the subject unable to undergo resistance training, or contraindication to biopsies (e.g. allergy to lidocaine). Subjects completed food frequency forms to determine typical diet and intake of dairy

products. Two groups were formed, CHO (n=9) and MILK (n=10), by matching groups for body weight and for their dairy intake to eliminate the possibility of bias. Subjects were asked to refrain from using any dietary supplements during the study without permission from an experimenter.

Design

Baseline, midpoint (after 5 weeks of training), and final testing were done to determine 1 RM for bench press, shoulder press, arm curl, lat pulldowns, leg press, leg curl, and leg extension. Subjects warmed up with approximately 10 RM, rested for 3 minutes, and increased resistance with rest in between attempts to reach 1 RM (data reported in thesis of Lauren Goldman).

Metabolic response to acute resistance exercise with beverage ingestion immediately afterwards was tested at least 2 days after determination of 1 RM for resistance exercises at baseline and after the 10-week resistance training program. Subjects followed meat-free diets for 4 days for these acute resistance exercise tests both before and after training: 3 days prior to the test of acute metabolic response, and the day of the test for acute metabolic response. Subjects completed 24-hour urine collections on the day before and the day of the acute test of metabolic response. Urine was collected in polypropylene bottles containing HCl.

Acute Metabolic Response to Resistance Exercise with Beverage Ingestion

Subjects reported to the laboratory for the test of acute metabolic response between 8:00 AM and 9:00 AM after a 12-hour fast. Muscle biopsies were performed within 30 minutes of starting exercise and at 1 hour after exercise. Muscle biopsies of the vastus lateralis were obtained before exercise and 1 hour after exercise. Biopsies were taken under local anesthesia with a hollow needle using suction in the same leg approximately 2 cm from each other. Biopsies taken at the end of the training program were taken from the leg that was not used for biopsies at baseline. Results from analysis of muscle biopsies are not included in this manuscript.

Resistance exercise consisted of 5 sets of 8 repetitions for leg press and leg extension at 80% of the subjects' 1 RM (determined prior to the test). Subjects were timed and supervised by the experimenters. Subjects rested for 2 minutes between sets of the same exercise, and 4 minutes rest was provided between the exercises.

Subjects were given a cool milk or carbohydrate-electrolyte beverage immediately after exercise, which was consumed within 5 minutes after starting consumption. Volume of the carbohydrate-electrolyte beverage was adjusted so that it was equal to the volume of a milk beverage with the same amount of calories.

Blood samples were taken from an arm vein before, immediately after, 30 minutes after, and 1 hour after exercise. Blood was centrifuged to separate the serum. The serum was frozen for later analysis of glucose, insulin, and IGF-1. Methanol and norleucine was added to the serum used for analysis of amino acids in a 2:1 ratio immediately after centrifugation, and the serum was refrigerated. This mixture of methanol and serum was centrifuged at high speed, at least 24 hours after the mixing of methanol and serum, and the supernatant was refrigerated for later analysis of amino acids.

Controlled Feeding Surrounding Exercise Test

Subjects were provided with specific meat-free menus for 2 days, while packages of meat-free diets were given to subjects to prepare on their own for the remaining 2 days of each 4-day period when the acute effect on metabolic response was measured. Protein sources included dairy, eggs, milk, and legumes. Subjects followed a planned diet for the 4-day period, and filled out a check sheet to note the foods that they consume or wasted.

Exercise Training

Subjects participated in a 10-week resistance exercise program, performing resistance exercise bouts at the Hokie Gym. The resistance training program was designed according to a program that recently caused significant increases in fat-free mass and muscle strength in young men over 10 weeks (9). Subjects trained 3 times per week, completing upper and lower body exercises at each workout. Resistance exercises

included bench press, shoulder press, arm curl, lat pulldowns, leg press, leg extension, and leg curl. The training period was split into 3 phases: 1) weeks 1-4: hypertrophy phase, 2) weeks 5-7: basic strength phase, 3) weeks 8-10: strength power phase. Workouts were performed according to the chart in Table 1.

Research assistants and graduate students instructed subjects on correct technique, and provided their supplement that was consumed immediately after exercise. A personal trainer was present at each session to ensure compliance with the program and proper technique for the exercises. The personal trainer also helped the subject complete a training log at each workout. As an incentive, subjects were given bonuses for attendance and strength gains up to $100 in addition to the $200 base for participation.

Supplements

Subjects were given their milk beverage or carbohydrate-electrolyte beverage after the resistance exercise workout by the graduate student, research assistant, or personal trainer. Beverages had 5 kcal/kg body weight, and were served cool. The milk beverage was chocolate low fat milk (0.92 g/kg carbohydrate, 0.21 g/kg protein, 0.06 g/kg fat). The carbohydrate-electrolyte beverage was Gatorade powdered sports drink mixed with water (1.25 g/kg of carbohydrate). The volume of the carbohydrate-electrolyte beverage provided was equal to that of a milk beverage with the same amount of calories.

Blood and Urine Analysis

Urinary 3-methylhistidine was analyzed by amino acid analyzer (PICO.TAG, Waters Association, Milford, MA) in the laboratory of Dr. Ken Webb in the Department of Animal and Poultry Sciences at Virginia Tech. Urinary creatinine was analyzed spectrophotometrically with a commercially available kit (Sigma #555).

Analyses of serum amino acids were conducted using an amino acid analyzer. Cysteine was not included in the total amino acid values reported in this study because it did not elute well, thus producing inaccurate measurements. Radioimmunoassay kits (DPC, Los Angeles, CA) were used to measure serum insulin, and IGF-I was measured

by radioimmunoassay according to the procedures described by Weber et al. (32). Duplicates of both insulin and IGF-1 were measured, with a coefficient of variation of 12.3% and 6.7%, respectively. Glucose was analyzed in duplicate using the spectrophotometric method (Sigma #315). The coefficient of variation for glucose analysis was 3.2%.

Sensory Data

Subjects rated their subjective response to their beverage immediately after consumption in the acute test. Subjects placed an "x" on a Likert scale 15 cm in length to evaluate the beverage with the far left side of the scale representing "not at all" and the far right, "completely" for four items: "Tastes good," "Quenches thirst," "Fulfills hunger," and "Energizing."

Statistical Analysis

The differences between groups for Likert scale results were analyzed by one-way ANOVA. Data for urinary measures, blood metabolites and hormones done before and after the acute resistance tests was analyzed with three-way repeated measures ANOVA (group, training status, pre/post resistance exercise). A mixed model technique was applied to account for 2 layers of correlation: 1) same subject used for multiple measurements and 2) multiple measurements of the same variable made in a short time period are correlated. A p value less than 0.05 was considered significant.

Results

Test of Acute Metabolic Response

Resistance exercise intensity was planned as 80% of the subjects' 1 RM, but some subjects were unable to complete the exercises at this resistance. Resistance was decreased for these subjects to the greatest resistance below 80% 1 RM that could be performed, and as a result average resistance completed was slightly less than 80% of the

subjects' 1 RM for some exercises. For CHO, leg press was completed at 79.7 +/- 0.1% of the subjects' 1 RM pre-training, and 80.0 +/- 0.1% post-training. Leg extension was performed at 75.6 +/- 0.7% of the subjects' 1 RM pre-training and 78.4 +/- 0.6% of the subjects' 1 RM post-training for CHO. MILK completed leg press at 78.1 +/- 0.6% of the subjects' 1 RM pre-training and 79.5 +/- 0.1% of the subjects' 1 RM post-training. Leg extension was performed at 75.7 +/- 0.8% and 80.3 +/- 0.8% of the subjects' 1 RM for MILK for pre-training and post-training, respectively.

Serum Amino Acids

Overall, serum concentrations of total amino acids, essential amino acids, and leucine were significantly higher for MILK than CHO. There was a significant time effect for serum essential amino acids, as concentration was higher pre-exercise (135.67 +/- 4.49 mg/L) than 1 hour post-exercise (127.64 +/- 4.56 mg/L). There was a significant group by time interaction for essential amino acids (Table 2). Essential amino acids decreased 25.8% for CHO from pre-exercise to 1 hour post-exercise, while concentration increased 12.4% for MILK from pre-exercise to 1 hour post-exercise.

Similar patterns and statistical results were seen for the concentration of serum leucine and total serum amino acids (Tables 3, 4). Total serum amino acid and serum leucine concentrations for CHO decreased 12.5% and 41.4% from pre-exercise to 1 hour post-exercise, respectively. Total serum amino acid concentration increased 15.2% and serum leucine concentration increased 9.9% for MILK from pre-exercise to 1 hour post-exercise. There was a significant time effect for serum leucine, as concentration was higher pre-exercise (20.82 +/- 0.76 mg/L) than 1 hour post-exercise (17.66 +/- 0.93 mg/L).

Serum Glucose

A significant effect of time was found for serum glucose, as concentration peaked at 39.3% above baseline at 30 minutes post-exercise, then dropped to 13.6% below baseline at 1 hour post-exercise (Figure 1). There was a significant group by time effect

for serum glucose, with greater increases in glucose at 30 minutes post-exercise for CHO compared to MILK (Figure 1). There was no effect of training on serum glucose response after resistance exercise.

Serum Hormones

A time effect was present for serum insulin ($p<0.0001$), as the concentration 30 minutes post-exercise was increased 7.2 times above baseline, then decreased at 1 hour post-exercise to a concentration 4.2 times greater than baseline. A group by time effect was found ($p<0.03$), as insulin was higher at 1 hour post-exercise in CHO than in MILK (Figure 2). There was no effect of training on serum insulin concentration.

There was a significant group effect for serum IGF-1 (Table 5). Overall, serum IGF-1 was higher for CHO (437.22 +/- 17.67 μg/L) than for MILK (363.95 +/- 13.39 μg/L). A significant time effect was also present for serum IGF-1, as concentration increased 3.5% from baseline to immediate post-exercise, then dropped 7.0% below baseline at 30 minutes post-exercise. There was no effect of training on serum IGF-1 response.

Urinary Metabolites

As a result of the failure of a number of subjects to completely collect urine on the day before and the day of the acute resistance tests, and an overfilling of the urine sample bottles by an experimenter (causing the bottles to burst, compromising the integrity of the sample), data for urinary metabolites does not include all subjects. Complete collections for all urinary measurements were analyzed for 6 subjects in CHO for the acute test before training and 7 subjects after training. All 10 subjects completely collected urine for 2 days for MILK for the acute test before training, but complete analyses for all urinary metabolites were only carried out for 5 subjects in MILK after training. Many subjects with incomplete data collected urine for at least 1 day for the acute tests. The statistical analysis program implemented analyzed the incomplete data for subjects by inserting dummy variables for the missing days.

A significant time effect was present for 3-methylhistidine excretion, as excretion on Day 2 (day of acute test) was decreased by 34.4% from Day 1 (day before acute test). There was also a significant time effect for the 3-methylhistidine-to-creatinine ratio, as the ratio dropped 19.9% from Day 1 (57.47 +/- 7.45 μmol 3-methylhistidine/g creatinine) to Day 2 (46.06 +/- 7.42 μmol 3-methylhistidine/g creatinine) (Figure 3). There was no effect of beverage for 3-methylhisitidine excretion or 3-methylhisitidine-to-creatinine ratio. No training effect was present for 3-methylhistidine excretion. A trend was present for a period by time effect for 3-methylhistidine-to-creatinine ratio ($p<0.07$). Reduction from Day 1 to Day 2 tended to be greater in post-training than in pre-training. There was no effect of beverage, training, or time for creatinine excretion.

Sensory Data

Subjects rated the taste of the milk beverage significantly higher than the carbohydrate-electrolyte beverage both before and after training (Table 6). There was no difference in subject rating for quenching of thirst, fulfillment of hunger, or energizing by beverage.

Discussion

The purpose of our study was twofold: 1) to determine the effect of consumption of a milk or carbohydrate-electrolyte beverage on muscle protein breakdown after an acute resistance exercise bout, and 2) to examine the effect of 10 weeks of resistance training on the acute response of muscle protein breakdown after resistance exercise with milk or carbohydrate-electrolyte consumption. Overall, muscle protein breakdown, estimated by 3-methylhistidine excretion and 3-methylhistidine-to-creatinine ratio, was significantly reduced after resistance exercise for subjects who consumed either the carbohydrate-electrolyte or milk supplements. The effects of the carbohydrate-electrolyte and milk supplements on muscle protein breakdown were not significantly different. The trend present for a training effect for 3-methylhistidine-to-creatinine ratio indicates that muscle protein breakdown was decreased with supplementation by a greater amount after

training. This trend was most evident for CHO, for which 3-methylhistidine-to-creatinine ratio was reduced by 16.2% after the exercise bout before training and 48.3% after training, but there was no significant interaction between beverage and training.

Although some researchers have criticized the use of 3-methylhistidine excretion to estimate protein breakdown, the procedure followed in this study is considered to be valid. Excretion was measured after 2 days of a meat-free diet, since 3-methylhistidine in the body is completely endogenous after this amount of time (18). The subject's diet was controlled for the 2 days before urine collection and for the 2 days when urine was collected. When diet is controlled, 3-methylhistidine excretion is considered to be an effective measure of muscle protein breakdown (10). Also, resistance exercise was strenuous, which has been reported by numerous research groups to cause an elevation in protein breakdown when a supplement is not provided (3, 14, 22, 30, 31). It can be assumed in our study that the contribution from nonskeletal muscle sources to 3-methylhistidine excretion is the same for all subjects, therefore the comparison of group results is an indication of the difference in skeletal muscle protein breakdown.

Because the subjects' 3-methylhistidine-to-creatinine ratio values in this experiment are much lower than those reported in previous research (15, 16, 24, 26, 27), these results are very questionable. Thus, this measurement may not be an accurate estimate of muscle protein breakdown. However, this may be due to the very large possible range for 3-methylhistidine excretion values. Previous researchers have reported these values for 3-methylhistidine-to-creatinine ratio at rest: approximately 260 μmol 3-methylhistidine/g creatinine for subjects in a study by Pivarnik et al. (24), compared to an average of 146 μmol 3-methylhistidine/g creatinine for Horswill et al. (16). These resting values are higher than exercise values for Roy et al. (26), Roy et al. (27), and our study (57.47 μmol 3-methylhistidine/g creatinine average for all subjects). Mean 3-methylhistidine-to-creatinine ratios after resistance exercise were approximately half of that reported in recent studies. For example, the average value was 40.09 μmol 3-methylhistidine/g creatinine after resistance exercise for CHO, compared to 110.43 μmol 3-methylhistidine/g creatinine (27) and 86.1 μmol 3-methylhistidine/g creatinine (26) after resistance exercise in previous studies with carbohydrate supplementation. Mean 3-

methylhistidine-to-creatinine ratio for MILK was 50.63 μmol 3-methylhistidine/g creatinine, compared to 84.2 μmol 3-methylhistidine/g creatinine in a study by Roy et al. (26) when carbohydrate/protein/fat supplement was provided after resistance exercise.

Low 3-methylhistidine measurements may have resulted from failure of the subjects to collect all of their urine, although expression relative to creatinine should reduce any effect of incomplete collection. Improper storage of urine by the subjects or experimenters may also have attributed to breakdown of 3-methylhistidine, leading to lower than normal measurements. Numerous checks of lab procedures and calculations did not show evidence of technical or calculation error. The abnormally low values for 3-methylhistidine excretion are possibly a limitation in our study.

The finding of a reduction in protein breakdown when a supplement is provided after resistance exercise is in agreement with results from studies by Rasmussen et al. (25) and Tipton et al. (29). In these studies, there was no change in protein breakdown from resting levels when an amino acid-carbohydrate supplement was ingested after exercise (25, 29). Results from our study differ from these results in that protein breakdown was reduced to levels below baseline after resistance exercise. Subjects in an experiment by Roy et al. (27) were given a carbohydrate supplement immediately after and 1 hour after resistance exercise. Compared to a placebo, 3-methylhistidine excretion (per gram of creatinine) was lower for the subjects given the carbohydrate supplement. Although protein breakdown was attenuated with supplementation after resistance exercise in the studies by Rasmussen et al. (25), Tipton et al. (29), and Roy et al. (27), the increases in protein balance that were present were mostly a result of an increase in protein synthesis.

Our research group measured 3-methylhistidine excretion for 2 days, the day before resistance exercise and the day of resistance exercise. Roy et al. (27) only measured 3-methylhistidine excretion on the day of resistance exercise. Unlike this study, Roy et al. (27) had no pre-exercise values for 3-methylhistidine excretion to determine if resistance exercise affected muscle protein breakdown. It is not possible to determine whether protein breakdown increased, decreased, or was not affected by resistance exercise. Roy et al. (27) concluded that the glucose supplement reduced

protein breakdown, but it is possible that pre-exercise 3-methylhistidine excretion was lower for the subjects before they were given the carbohydrate supplement.

Rasmussen et al. (25) and Tipton et al. (29) reported that protein breakdown was no changed from resting with supplementation of an amino acid-carbohydrate beverage by measuring isotope infusion. Isotope infusion may be a more sensitive and reliable measurement of protein breakdown than 3-methylhistidine excretion. Yarasheski et al. (34) demonstrated this possibility by measuring protein breakdown after resistance exercise with both isotope infusion and 3-methylhistidine excretion. Isotope infusion revealed an increase in protein breakdown, but there was no change in 3-methylhistidine excretion (34). In some studies, 3-methylhistidine excretion was decreased (21) or unchanged (15, 16) after intense resistance exercise. Researchers using isotope infusion, on the other hand, have found an increase in protein breakdown after intense resistance exercise (3, 22, 30). Since the sensitivity of 3-methylhistidine as a measure of muscle protein breakdown is unknown, our study may have benefited from the use of a more reliable lab procedure in place of or in combination with measurement of 3-methylhistidine excretion.

One study has been done to compare the effect on protein breakdown after resistance exercise of a carbohydrate supplement to a mixture of carbohydrate, fat and protein (26). Roy et al. (26) concluded, by measuring 3-methylhistidine excretion (per gram of creatinine), that neither supplement was effective in reducing protein breakdown when compared to a placebo, and there were no significant differences between the treatments. Roy et al. (26) stated that 3-methylhistidine excretion was decreased in this experiment by the carbohydrate and carbohydrate/protein/fat supplements when compared to the placebo, but not by a significant amount. Similar to our study, both the carbohydrate and carbohydrate/protein/fat supplements caused an increase in blood glucose and significant hyperinsulinemia. Hyperaminoacidemia was present for the group given the carbohydrate/protein/fat supplement, but not the carbohydrate or placebo supplements. Since the environment in the serum after resistance exercise seemed favorable for reduction of protein breakdown, the lack of significant results is not easy to explain.

Similar to his previous study (27), Roy et al. (26) only measured 3-methylhistidine-to-creatinine ratio on the day of resistance exercise, which makes a conclusion about the effects on muscle protein breakdown difficult. It is possible that muscle protein breakdown was decreased by the treatments, but that the measurement of 3-methylhistidine excretion was not sensitive enough to detect this effect. Roy et al. (26) stated that the reduction in protein breakdown may be "clinically relevant," and the research group believed that this trend for a reduction in protein breakdown was important. However, a difference in the protein breakdown for the carbohydrate drink when compared to the carbohydrate/protein/fat drink was not apparent.

The fact that we did not include a control or placebo group limits our ability to make conclusions about the effect of the resistance exercise on muscle protein breakdown. However, in a preliminary study carried out in our laboratory (Bird, unpublished results), muscle protein breakdown was reduced in subjects consuming placebo, carbohydrate-electrolyte beverage, or milk beverage after performing the same exercise bout that was implemented in this study. A concern about interpretation of that data was the brief meat-free diet (2 days as opposed to 4 days in this study). However, neither study performed in our lab with this exercise bout resulted in an increase in muscle protein breakdown, regardless of beverage ingestion.

Consumption of the milk or carbohydrate-electrolyte supplement may have reduced protein breakdown after resistance exercise through creation of a hormonal environment in the serum associated with improved protein balance. An effect induced by both beverages, hyperinsulinemia, may reduce protein breakdown by stimulating amino acid transport (1). The supplements induced hyperinsulinemia secondary to increased serum glucose concentration. Hyperaminoacidemia, which was present for MILK, may decrease protein breakdown by increasing amino acid availability (1).

The alterations in insulin concentration for CHO and MILK were similar to those seen in studies with subjects that consumed an amino acid-carbohydrate (25, 29) or carbohydrate (27) supplement after resistance exercise. The supplements increased serum glucose, which caused a peak in insulin concentration 30 minutes after drink consumption, followed by reduction to a concentration significantly greater than baseline.

The anabolic effects demonstrated with amino acid-carbohydrate (25) or carbohydrate (27) supplementation may have been aided by an increase in amino acid availability and a stimulation of amino acid transport by hyperinsulinemia.

An important difference in the metabolic response to the drinks was the effect on serum amino acids. Supplementation of the milk drink resulted in significant hyperaminoacidemia, as seen in previous studies using an amino acid-carbohydrate supplement with resistance training by Rasmussen et al. (25) and Tipton et al. (29). Rasmussen et al. (25) and Tipton et al. (29) did not measure serum concentration of total amino acids, but determined that hyperaminoacidemia was present by measuring phenylalanine concentration. Hyperaminoacidemia increases amino acid availability, which may decrease muscle protein breakdown (25). Hyperaminoacidemia, along with hyperinsulinemia, resulting from the amino acid-carbohydrate supplement may have aided in the reduction of protein breakdown present after resistance exercise (25). In contrast to the effects of the milk beverage, the carbohydrate-electrolyte beverage caused a reduction in serum amino acids.

Roy et al. (27), Rasmussen et al. (25), and Tipton et al. (29) did not compare the effects of a carbohydrate supplement to an amino acid-carbohydrate supplement. Roy et al. (26) compared the effect on protein breakdown after resistance exercise of a carbohydrate supplement to a carbohydrate/protein/fat supplement. Similar to our study, the effect on protein breakdown was not different between the supplements. However, neither supplement in the study by Roy et al. (26) was beneficial for reducing protein breakdown compared to a placebo. More research is needed to compare the effect on protein breakdown of a carbohydrate supplement to a carbohydrate/protein/fat supplement.

In studies involving an amino acid-carbohydrate supplement (25, 29), it was assumed that the beverage reduced protein breakdown after resistance exercise because there was no elevation of protein breakdown above resting levels. There was no group in either of these studies that only received a placebo (no supplementation at any time near the exercise period), thus it cannot be verified that the beverages caused a reduction in protein breakdown (25, 29). Although previous researchers have noted that an increase in

protein breakdown is present in the fasted state (3, 14, 22, 30, 31), an elevation in protein breakdown after resistance exercise was not present in recent studies (26, 28). Thus, it may not be safe to assume that a lack of elevation from resting after resistance exercise represents a reduction in protein breakdown. In the future, researchers need to include a group that is not given any supplements in order to determine if protein breakdown is increased after resistance exercise.

Since an amino acid-carbohydrate supplement causes both hyperaminoacidemia and hyperinsulinemia, and a carbohydrate supplement causes a reduction in serum amino acid concentration, it may be hypothesized that the amino acid-carbohydrate supplement would be more effective in reduction of protein breakdown. Researchers support this notion; while protein breakdown has been reduced significantly by hyperinsulinemia (5, 8, 12, 13, 19, 27) and hyperaminoacidemia (4), combining these states has been more beneficial in decreasing protein breakdown than either state alone (2, 11). However, our results did not support this in that protein breakdown for CHO was similar to that for MILK in spite of opposite effects on serum amino acids. One explanation for this finding may be the significantly greater hyperinsulinemia for CHO compared to MILK, allowing for further stimulation of amino acid transport and reduced muscle protein breakdown. Fukagawa et al. (12) demonstrated that protein breakdown, as estimated by plasma 3-methylhistidine, was decreased for subjects as the insulin dose was increased. A greater degree of hyperinsulinemia may have balanced the reduction in serum amino acids for CHO.

Serum IGF-1 was increased for MILK and CHO compared to baseline concentration immediately after resistance exercise in our study, and was reduced below baseline at 1 hour post-exercise. These results are unlike those of previous studies with supplementation after resistance exercise by Kraemer et al. (17) and Chandler et al. (7). Kraemer et al. (17) reported that there were no acute alterations in IGF-1 concentration after resistance exercise when subjects were given a protein-carbohydrate supplement before and immediately after resistance exercise. IGF-1 concentrations were not changed after resistance exercise in a study by Chandler et al. (7) when a protein, carbohydrate, or protein-carbohydrate supplement was given. Although IGF-1 concentrations were

significantly altered after acute resistance exercise in our study, the percent changes in concentration were small and most likely not clinically significant.

IGF-1 concentration was not changed after resistance exercise in a study by Nindl et al. (20), but there were alterations in IGF binding protein concentrations. Nindl et al. (20) concluded that changes in the IGF-1 system may affect strength gains associated with resistance exercise. IGF binding protein concentrations were not measured in our study. The acute changes in IGF-1 concentration with resistance exercise in our study may have been accompanied by alterations in IGF binding protein concentrations, leading to a significant influence on strength gains. The relevance of acute alteration in IGF-1 concentration after resistance exercise is not completely established. Therefore, it is difficult to conclude if changes in IGF-1 concentration contributed to the reduction in protein breakdown after resistance exercise. Beverage did not influence IGF-1 concentration. This is in contrast to the study by Kraemer et al. (17), in which resting IGF-1 was elevated on the second and third days after supplementation with a protein-carbohydrate beverage compared to a placebo. More research needs to be done to determine the effect of supplementation on acute changes in IGF-1 concentration with resistance exercise, and the possible implications for muscle protein breakdown and subsequent strength gains.

Previous research has not been done to examine the effect of training on the protein breakdown response to resistance training with supplementation. Our research group discovered a trend for greater reduction in protein breakdown after resistance exercise when our subjects were in the trained state compared to the start of the study when they were untrained. The subjects in our study were considered to be in the trained state for the second acute test, as there was an overall strength increase of 44% over the 10-week training program, with no difference between the groups (reported in thesis of Lauren Goldman). Other researchers support a lower muscle protein breakdown after resistance exercise in trained individuals (23, 30).

In conclusion, muscle protein breakdown was significantly reduced after a strenuous resistance exercise bout with consumption of a milk or carbohydrate-electrolyte supplement, as estimated by 3-methylhistidine excretion and 3-methylhistidine-to-

creatinine ratio, with no difference between the treatments. This lack of difference between beverages is similar to the results from the only other published study that compared a carbohydrate to carbohydrate/protein/fat beverage after resistance exercise (26). Both hyperinsulinemia and hyperaminoacidemia were induced by the milk supplement, but hyperaminoacidemia was not present for CHO. CHO had a significantly greater degree of hyperinsulinemia, which may have allowed for similar reduction in protein breakdown between the groups. We can conclude from our research that consumption of a milk beverage after resistance exercise appears to have similar effects on protein breakdown as a carbohydrate-electrolyte beverage. Resistance training reduced the muscle protein breakdown response to an acute resistance exercise bout with no difference in this effect by beverage ingestion post-exercise.

Since research comparing carbohydrate and carbohydrate/protein/fat (such as milk) supplements is limited, further studies are needed to determine which beverage is more effective for reduction of protein breakdown. Addition of a control group will help researchers to conclude if the alteration in protein breakdown after resistance exercise is caused by the milk or carbohydrate supplement. The use of isotope infusion may provide a more reliable estimate of protein breakdown. Finally, because the degree of hyperinsulinemia present affects muscle protein breakdown, research should be done to determine the optimal amount of carbohydrate to provide after resistance exercise for elevation of serum insulin concentration.

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Table 1: Description of Workouts Performed in 10 week training period

Week	Sets	Repetitions	% of 1 RM
1	3	12	55%
2	4	10	64%
3	4	10	67%
4	4	8	70%
5	4	6	73%
6	4	6	76%
7	5	5	82%
8	5	5	85%
9	3	3	94%
10	3	3	97%

Table 2: Serum essential amino acid concentration (mg/L) before and after 10 weeks of resistance training

	Pre-Training	
	Pre-Exercise	**1 Hour Post**
CHO*	143.29	109.21
(n=8)	(2.64)	(3.83)
MILK	123.97	143.14
(n=10)	(14.80)	(4.16)
	Post-Training	
	Pre-Exercise	**1 Hour Post**
CHO*	138.46	100.39
(n=9)	(4.51)	(2.38)
MILK	137.99	151.42
(n=10)	(6.63)	(8.66)

Values are averages with SEM in parentheses
1 sample was missed for CHO pre-training, thus n is one less at this time point
the statistical program used inserted variables for missing data
*indicates significant group by time interaction

Table 3: Serum leucine concentration (mg/L) before and after 10 weeks of resistance training

	Pre-Training	
	Pre-Exercise	**1 Hour Post**
CHO*	22.14	12.66
(n=8)	(0.61)	(0.62)
MILK	18.34	20.43
(n=10)	(2.34)	(0.92)
	Post-Training	
	Pre-Exercise	**1 Hour Post**
CHO*	21.86	13.11
(n=9)	(0.71)	(0.47)
MILK	21.18	22.99
(n=10)	(1.24)	(1.69)

Values are averages with SEM in parentheses
1 sample was missed for CHO pre-training, thus n is one less at this time point
the statistical program used inserted variables for missing data
*indicates significant group by time interaction

Table 4: Serum total amino acid concentration (except cysteine) (mg/L) before and after 10 weeks of resistance training

	Pre-Training	
	Pre-Exercise	**1 Hour Post**
CHO*	367.80	339.09
(n=8)	(7.05)	(11.67)
MILK	329.95	400.74
(n=10)	(37.09)	(11.47)

	Post-Training	
	Pre-Exercise	**1 Hour Post**
CHO*	363.06	302.44
(n=9)	(15.67)	(8.17)
MILK	372.61	408.73
(n=10)	(13.63)	(17.07)

Values are averages with SEM in parentheses
1 sample was missed for CHO pre-training, thus n is one less at this time point
the statistical program used inserted variables for missing data
*indicates significant group by time interaction

Table 5: Serum IGF-1 concentration (μg/L) before and after 10 weeks of resistance training

Pre-Training

	Pre-Exercise**	Immediate Post	30 minutes Post	1 Hour Post
CHO*	448.54	467.37	397.44	434.14
(n=9)	(50.20)	(33.25)	(33.61)	(58.48)
MILK	354.33	338.69	347.52	324.98
(n=10)	(35.32)	(37.08)	(24.25)	(25.32)

Post-Training

	Pre-Exercise**	Immediate Post	30 minutes Post	1 Hour Post
CHO*	456.39	490.99	419.50	383.46
(n=9)	(44.05)	(69.89)	(58.56)	(47.85)
MILK	384.96	406.27	362.78	392.10
(n=10)	(42.75)	(47.57)	(43.21)	(44.83)

Values are averages with SEM in parentheses
*indicates overall values for CHO significantly greater than MILK at $p<0.05$
**indicates significant time effect at $p<0.05$

Table 6: Likert Scale Results for Post-Exercise Beverage

Tastes Good

	Pre-Training	Post-Training
CHO (n=9)	7.1 (1.2)	7.6 (1.4)
MILK* (n=10)	11.1 (0.8)	11.3 (0.6)

Quenches Thirst

	Pre-Training	Post-Training
CHO (n=9)	8.4 (0.9)	8.8 (0.9)
MILK (n=10)	6.7 (0.9)	8.3 (1.1)

Fulfills Hunger

	Pre-Training	Post-Training
CHO (n=9)	7.5 (0.4)	7.4 (0.5)
MILK (n=10)	8.0 (1.3)	9.3 (0.9)

Energizing

	Pre-Training	Post-Training
CHO (n=9)	8.2 (0.7)	7.2 (1.0)
MILK (n=10)	6.2 (0.9)	6.4 (1.3)

Values are distance from the left end of scale (cm) on 15 cm scale, from "not at all" on far left to "completely" on far right
Values are averages with SEM in parentheses

*indicates significant difference between groups at $p<0.05$

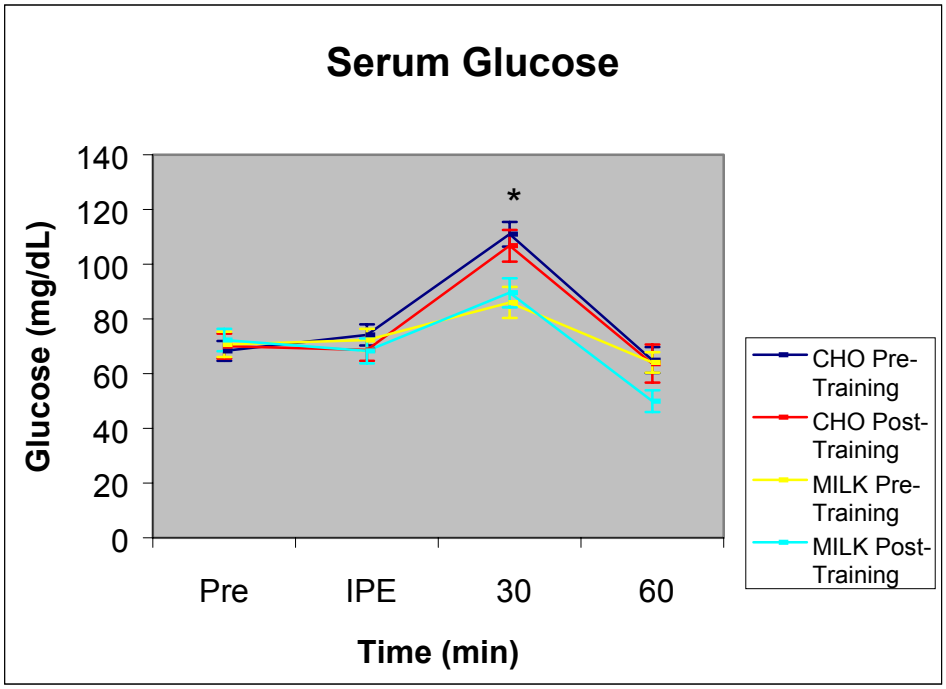

Figure 1: Serum glucose concentration

Pre = Pre-Exercise; IPE = Immediate Post-Exercise

* indicates overall significant difference at this time point between CHO and MILK at $p<0.05$

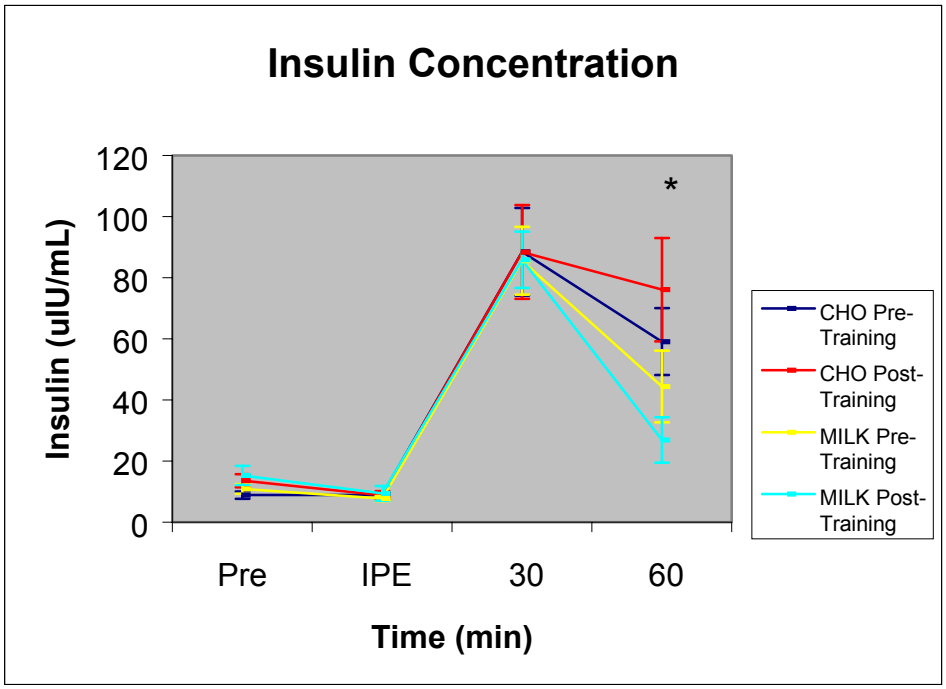

Figure 2: Serum insulin concentration

Pre = Pre-Exercise; IPE = Immediate Post-Exercise

* indicates overall significant difference at this time point between CHO and MILK at $p<0.05$

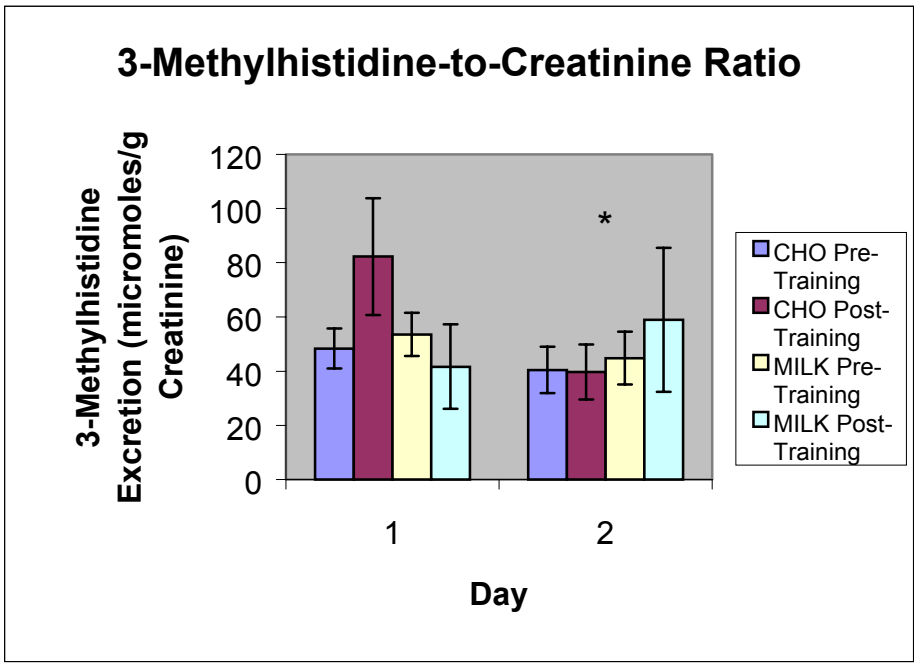

Figure 3: 3-Methylhistidine-to-creatinine ratio

* indicates overall significant difference between Day 1 and Day 2 at $p<0.05$

CHAPTER 4: SUMMARY AND RECOMMENDATIONS

Many researchers have discovered an increase in muscle protein breakdown after strenuous resistance exercise (5, 23, 37, 46, 47). This elevation in muscle protein breakdown has been shown to be significantly lower for trained subjects (38, 46). As a result, trained individuals may have a more positive protein balance after resistance exercise, which allows for greater increases in muscle size and strength.

Determination of a method to reduce protein breakdown after resistance exercise would be beneficial for creation of a more positive muscle protein balance. Recently, researchers have studied the effects of carbohydrate and protein supplementation on protein breakdown. Biolo et al. (6) reported that amino acid infusion after an acute resistance exercise bout significantly decreased muscle protein breakdown. This attenuation of protein breakdown is most likely the result of increased amino acid availability via hyperaminoacidemia (1). Tipton et al. (44) determined that essential amino acids are as effective as a mixture of all amino acids in enhancing muscle protein balance after resistance exercise. Thus, nonessential amino acids do not appear to be an important ingredient for supplementation.

Carbohydrate may aid in reduction of protein breakdown after resistance exercise. In a study by Roy et al. (43), a carbohydrate supplement reduced protein breakdown after a resistance exercise bout, probably through stimulation of amino acid transport with hyperinsulinemia (1). Supplements containing a combination of carbohydrate and amino acids have also been beneficial for decreasing protein breakdown after resistance exercise (40, 45).

A beverage with both carbohydrate and amino acids may be the most effective supplement for reduction of protein breakdown after resistance exercise, since it induces both hyperaminoacidemia and hyperinsulinemia. One research group has compared the effect of a carbohydrate beverage to a carbohydrate/protein/fat mixture on protein breakdown after resistance exercise (42). Roy et al. (42) concluded that neither supplement had an effect on protein breakdown after resistance exercise. Since the reliability of the method used to estimate protein breakdown in this experiment, 3-methylhistidine excretion, is not known, it is difficult to make a conclusion about the effects on muscle protein breakdown with supplementation. Therefore, more research is

needed to compare the effect on protein breakdown after resistance exercise of a carbohydrate/protein/fat supplement to a carbohydrate beverage.

Milk may be an effective beverage for reduction of muscle protein breakdown when consumed after resistance exercise. Milk is naturally occurring, and is made up of a mixture of carbohydrate, protein, and fat; nutrients associated with attenuation of protein breakdown. The protein in milk is high quality, and it consists of many essential amino acids, protein components proven to enhance muscle protein balance after resistance exercise. Very little research has been completed to determine the effect of a milk beverage on protein breakdown after resistance exercise. Wojcik et al. (49) reported that a milk beverage fed after strenuous eccentric exercise tended to reduce 3-methylhistidine excretion compared to carbohydrate or placebo, but these results were not significant. Since the constituents of milk have been associated with a reduction in protein breakdown after resistance exercise, additional research with this beverage is warranted.

The purpose of our study was twofold: 1) to determine the effect of consumption of a milk or carbohydrate-electrolyte beverage on muscle protein breakdown after an acute resistance exercise bout, and 2) to examine the effect of 10 weeks of resistance training on the acute response of muscle protein breakdown after resistance exercise with milk or carbohydrate-electrolyte consumption.

Nineteen untrained males between the ages of 18-25 years took part in this study. The subjects had not participated in resistance training for at least 3 months prior to the start of the study. Subjects were placed into the CHO (n=9) or MILK (n=10) group, with matching for body weight and dairy intake. Metabolic response to acute resistance exercise was determined before and after 10 weeks of resistance training. In the acute tests, subjects completed 5 sets of 8 repetitions for leg press and leg extension, both at or near 80% 1 RM. Blood samples were taken for analysis of serum metabolites and hormones, and urine was collected the day before and the day of the acute test for measurement of creatinine and 3-methylhistidine excretion. Subjects consumed a controlled, meat-free diet for 3 days before the acute tests and the day of the acute tests so that the measurement of 3-methylhistidine excretion would be valid.

Despite limited data resulting from failure of some subjects to completely collect

urine and overfilling of urine sample bottles by an experimenter (causing them to burst, compromising the integrity of the sample), a significant time effect was present for 3-methylhistidine excretion, as excretion on Day 2 (the day of the acute test) was decreased by 34.4% from Day 1 (the day before the acute test). There was also a significant time effect for 3-methylhistidine-to-creatinine ratio, as the ratio dropped 19.9% from Day 1 to Day 2. A trend was present for a training effect for 3-methylhistidine-to-creatinine ratio ($p<0.07$). Reduction from Day 1 to Day 2 tended to be greater in post-training than in pre-training. There were no differences between beverages for 3-methylhistidine excretion and 3-methylhistidine-to-creatinine ratio.

Serum glucose was increased for both groups at 30 minutes post-exercise. However, this elevation was significantly greater for CHO. This increase allowed for significantly higher insulin concentrations for CHO than MILK at 1 hour post-exercise. Insulin concentration peaked 30 minutes post-exercise at about 7 times the baseline concentration, then dropped to a concentration at 1 hour post-exercise that was still significantly higher than baseline for both groups.

The decrease in 3-methylhistidine excretion and 3-methylhistidine-to-creatinine ratio after resistance exercise indicates a reduction in protein breakdown with the carbohydrate-electrolyte or milk supplement. The finding of a reduction in protein breakdown when a supplement is provided after resistance exercise is in agreement with results from studies providing carbohydrate (43), and a mixture of carbohydrate and amino acids (40, 45) after resistance exercise.

Hyperinsulinemia was most likely instrumental for both groups for reduction of protein breakdown by stimulating amino acid transport (1). Hyperaminoacidemia, present only for MILK, also may decrease muscle protein breakdown by increasing amino acid availability (1). Since both hyperaminoacidemia and hyperinsulinemia were present for MILK, it may be predicted that protein breakdown would be reduced more with the milk supplement. However, the supplements were equally effective in attenuating protein breakdown. This may be because hyperinsulinemia was significantly greater for CHO, allowing for further stimulation of amino acid transport. The elevation in serum glucose was larger for CHO 30 minutes post-exercise, which allowed for higher insulin

concentrations 1 hour after exercise. The more advanced hyperinsulinemia present for CHO may have made up for the lack of hyperaminoacidemia, thus resulting in a reduction in protein breakdown similar to that for MILK.

The trend present for a training effect for 3-methylhistidine-to-creatinine ratio indicates that muscle protein breakdown was decreased by a greater amount with supplementation after training. Other researchers support a lower muscle protein breakdown after resistance exercise in trained individuals (38, 46).

Recommendations for Future Research

1. In our study, there was not a control group to determine the degree of protein breakdown present when a placebo is consumed. We concluded that the milk or carbohydrate-electrolyte beverage caused the reduction in 3-methylhistidine-to-creatinine ratio and 3-methylhistidine excretion after resistance exercise when compared to pre-exercise values. An elevation in 3-methylhistidine-to-creatinine ratio and 3-methylhistidine excretion after resistance exercise for a control group would strengthen this statement. This would help in future studies to determine if the carbohydrate or milk supplement is the cause of a reduction in protein breakdown after resistance exercise.

2. Previous researchers have indicated that the measurement of 3-methylhistidine is an inconsistent method for estimation of muscle protein breakdown (24, 25, 36, 50). Isotope infusion has provided more consistent results for muscle protein breakdown (5, 37, 46, 50). In the future, researchers comparing the effects on protein breakdown of milk and carbohydrate supplementation may produce more accurate results with use of isotope infusion. It would also be worthwhile to determine the sensitivity of 3-methylhistidine as a marker for protein breakdown. This may be done by measuring muscle protein breakdown after resistance exercise via both isotope infusion and 3-methylhistidine excretion.

3. Our research group measured protein breakdown for the first 24 hours after resistance

exercise. Previous researchers reported that muscle protein breakdown is elevated for up to 48 hours after exercise (37, 46). In order to determine the complete magnitude of muscle protein breakdown after resistance exercise, researchers may need to record measurements for 2 days after exercise.

4. Two strenuous lower body resistance exercises were done in the acute tests in this study. Although muscle protein breakdown may increase significantly after completion of numerous sets of 1 strenuous resistance exercise (37), completion of several different strenuous resistance exercises, both upper and lower body, may result in a greater amount of protein breakdown. This would help to ensure a significant increase in muscle protein breakdown regardless of training status, and aid in the distinction between treatment and control groups, if in fact the treatment aids in reduction of protein breakdown. This also will provide results that are applicable to normal training, since most resistance exercise bouts consist of several different exercises.

5. The effects of a milk and carbohydrate-electrolyte beverage on protein breakdown were compared in this study. Amino acid infusion after resistance exercise has also decreased protein breakdown (6). Therefore, the effect of supplementation with protein or amino acids alone on muscle protein breakdown should be compared in the same study to the effects of a carbohydrate or milk beverage.

6. The degree of hyperinsulinemia has been shown to affect muscle protein breakdown (20). The degree of hyperaminoacidemia present after resistance exercise may also affect protein breakdown. In the future, researchers should compare the effects of supplementation with different amounts of amino acids and carbohydrate on muscle protein breakdown after resistance exercise. This will aid in determination of the optimal amount of each macronutrient to provide after resistance exercise.

Appendix A: Detailed Description of Research Methods and Procedures

Subject Selection and Screening

Twenty-one males between the ages of 18-25 years were selected for this study, but 2 subjects dropped out of the study for health reasons. The subjects were not taking any nutritional supplements other than a multivitamin and had not been involved in resistance training for 3 months before the start of the study.

Subjects completed a health history questionnaire to screen for any contraindications to strenuous resistance exercise, such as skeletal injuries or cardiovascular problems. The questionnaire also screened subjects for food or lidocaine allergies and lactose intolerance. Informed consent forms were read and signed by all the subjects.

Subject Pre-Testing

Subjects' 1 RM for leg press and leg extension were determined at least 2 days prior to the acute test of metabolic response both before and after the 10-week resistance training program. Subjects first completed 10 repetitions at a light weight to warm up and become acclimated to the equipment. Subjects then performed trials at progressively higher resistances, with a rest period of 1.5 minutes in between each trial, until a resistance was reached that they could only complete once.

Urine Samples

Subjects completed 24-hour urine collections for the day before and the day of the acute test of metabolic response both before and after the 10-week resistance training program. Samples were collected in 1 L polypropylene bottles with 1 mL hydrochloric acid to make sure that ammonia did not become volatile. Collections began with the second void of the day and were brought to the lab the next morning. The daily samples for each subject were mixed and total volume was recorded. Then 5 mL samples were frozen for later analysis of creatinine and 3-methylhistidine.

Urine samples were thawed at room temperature, and creatinine concentration was analyzed in duplicate spectrophotometrically according to directions for a commercially

available kit (Kit #555, Sigma Chemical Co., St. Louis, MO). Duplicates with greater than 10% difference were reanalyzed. Urinary 3-methylhistidine was analyzed by amino acid analyzer (PICO.TAG, Waters Association, Milford, MA) in the laboratory of Dr. Ken Webb in the Department of Animal and Poultry Sciences at Virginia Tech.

Blood Collection and Analysis

Blood samples were taken from an arm vein by a certified clinical laboratory technician during the acute test of metabolic response. Blood samples were taken before exercise (fasted) as well as immediately, 30 minutes, and 60 minutes after exercise. Blood samples were placed in an ice bath for 30 minutes to clot and were centrifuged at 3,000 rpm for 15 minutes at 4°C. Aliquots of serum were stored at -20°C for later analysis. Methanol containing norleucine was added to 1 mL of serum in duplicate for analysis of amino acids in a 2:1 ratio immediately after centrifugation, and the serum was refrigerated. This mixture of methanol and serum was centrifuged at 10,500 rpm for 20 minutes at 4°C at least 24 hours after the mixing of methanol and serum, and the supernatant was refrigerated for later analysis of amino acids. Serum amino acids were analyzed in duplicate by amino acid analyzer (PICO.TAG, Waters Association, Milford, MA) in the laboratory of Dr. Ken Webb in the Department of Animal and Poultry Sciences at Virginia Tech. Cysteine was not included in the total amino acid values reported in this study because it did not elute well, thus producing inaccurate measurements.

Serum was thawed at room temperature and analyzed in duplicate to determine serum concentrations of glucose, insulin, and IGF-1. Glucose was analyzed spectrophotometrically according to directions for a commercially available kit (Kit #315, Sigma Chemical Co., St. Louis, MO). Duplicates with greater than a 10% difference were reanalyzed. Coat-a-Count radioimmunoassay kits (Diagnostic Products Corp, Los Angeles, CA) were used to analyze serum insulin. Any duplicates with greater than a 25% difference between samples were reanalyzed. Serum IGF-1 was analyzed by radioimmunoassay as described by Weber et al. (48), and duplicates with a difference greater than 25% were reanalyzed.

Acute Exercise Bout

Subjects completed 5 sets of 8 repetitions for leg press and leg extension both at approximately 80% 1 RM in the acute test of metabolic response before and after resistance training. Some subjects could not complete 5 sets at 80% 1 RM for the exercises, so the resistance was decreased slightly to a weight that the subject could lift (Appendix B). Subjects rested for 2 minutes between sets for each exercise, and for 4 minutes between exercises.

Nutritional Supplementation

Subjects completed a dairy frequency checklist and were placed into groups to match dairy intake (presented in thesis of Lauren Goldman). Subjects were given their milk beverage or carbohydrate-electrolyte beverage within 5 minutes after resistance exercise in the acute tests. Beverages had 5 kcal/kg body weight, and were served cool. The beverages were consumed in less than 5 minutes. The milk beverage was chocolate low fat milk (0.92 g/kg carbohydrate, 0.21 g/kg protein, 0.06 g/kg fat). The carbohydrate-electrolyte beverage was Gatorade powdered sports drink mixed with water (1.25 g/kg of carbohydrate). The volume of the carbohydrate-electrolyte beverage provided was equal to that of a milk beverage with the same amount of calories.

Controlled Feeding Surrounding Exercise Test

Subjects were provided with specific meat-free menus for 2 days, while packages of meat-free diets were given to subjects to prepare on their own for the remaining 2 days of each 4-day period when the acute effect on metabolic response was measured. The diets provided 30 kcal/kg body weight. Subjects followed a planned diet for the 4-day period, and filled out a check sheet to note the foods that they consume or wasted.

Appendix B: Individual Values for Lab Measurements

Table 1: CHO Pre-Training Acute Exercise Bout

Subject	Set	Leg Press Acute test (lbs)	Leg Press % of 1 RM	Leg Extension Acute test (lbs)	Leg Extension % of 1 RM
1	1	375	80%	250	81%
	2	375	80%	230	74%
	3	375	80%	200	65%
	4	375	80%	200	65%
	5	375	80%	200	65%
3	1	245	79%	150	79%
	2	245	79%	140	74%
	3	245	79%	140	74%
	4	245	79%	140	74%
	5	245	79%	140	74%
5	1	600	80%	150	77%
	2	600	80%	150	77%
	3	600	80%	150	70%
	4	600	80%	150	70%
	5	600	80%	150	70%
12	1	295	80%	175	80%
	2	295	80%	175	80%
	3	295	80%	175	80%
	4	295	80%	175	80%
	5	295	80%	175	80%
13	1	150	79%	135	79%
	2	150	79%	135	79%
	3	150	79%	130	76%
	4	150	79%	130	76%
	5	150	79%	130	76%
14	1	360	80%	200	80%
	2	360	80%	200	80%
	3	360	80%	200	80%
	4	360	80%	200	80%
	5	360	80%	200	80%
15	1	215	80%	125	78%
	2	215	80%	125	78%
	3	215	80%	125	78%
	4	215	80%	125	78%
	5	215	80%	125	78%
18	1	230	79%	120	75%
	2	230	79%	120	75%
	3	230	79%	120	75%
	4	230	79%	120	75%
	5	230	79%	120	75%
21	1	350	80%	220	77%
	2	350	80%	220	77%
	3	350	80%	200	70%
	4	350	80%	200	70%
	5	350	80%	200	70%

Table 2: CHO Post-Training Acute Exercise Bout

Subject	Set	Leg Press Acute test (lbs)	Leg Press % of 1 RM	Leg Extension Acute test (lbs)	Leg Extension % of 1 RM
1*	1	290	81%	145	81%
	2	290	81%	145	81%
	3	290	81%	145	81%
	4	290	81%	145	81%
	5	290	81%	145	81%
3	1	380	79%	230	81%
	2	380	79%	230	81%
	3	380	79%	230	81%
	4	380	79%	230	81%
	5	380	79%	230	81%
5***	1	600	80%	150	68%
	2	600	80%	150	68%
	3	600	80%	150	68%
	4	600	80%	150	68%
	5	600	80%	150	68%
12	1	490	80%	260	80%
	2	490	80%	260	80%
	3	490	80%	260	80%
	4	490	80%	260	80%
	5	490	80%	260	80%
13	1	360	80%	220	80%
	2	360	80%	220	80%
	3	360	80%	220	80%
	4	360	80%	220	80%
	5	360	80%	220	80%
14	1	455	80%	235	80%
	2	455	80%	235	80%
	3	455	80%	235	80%
	4	455	80%	235	80%
	5	455	80%	235	80%
15	1	512	80%	207.5	80%
	2	512	80%	207.5	80%
	3	512	80%	207.5	80%
	4	512	80%	207.5	80%
	5	512	80%	207.5	80%
18**	1	185	80%	207.5	80%
	2	185	80%	207.5	80%
	3	185	80%	207.5	80%
	4	185	80%	207.5	80%
	5	185	80%	207.5	80%
21	1	530	80%	255	80%
	2	530	80%	240	75%
	3	530	80%	240	75%
	4	530	80%	240	75%
	5	530	80%	240	75%

*denotes use of 1 leg for leg press and leg extension

**denotes use of one leg for leg press
***denotes use of one leg for leg extension

Table 3: MILK Pre-Training Acute Exercise Bout

Subject	Set	Leg Press Acute test (lbs)	Leg Press % of 1 RM	Leg Extension Acute test (lbs)	Leg Extension % of 1 RM
2	1	430	80%	220	77%
	2	430	80%	200	70%
	3	410	67%	200	70%
	4	360	67%	200	70%
	5	360	67%	200	70%
4	1	430	80%	180	78%
	2	430	80%	180	78%
	3	430	80%	180	78%
	4	430	80%	180	78%
	5	430	80%	180	78%
6	1	240	80%	168.8	80%
	2	240	80%	168.8	80%
	3	240	80%	168.8	80%
	4	240	80%	168.8	80%
	5	240	80%	168.8	80%
7	1	205	79%	210	76%
	2	205	79%	210	76%
	3	205	79%	210	76%
	4	205	79%	210	76%
	5	205	79%	210	76%
10	1	295	80%	220	68%
	2	295	80%	220	68%
	3	295	80%	220	68%
	4	295	80%	220	68%
	5	295	80%	220	68%
11	1	440	80%	216.6	80%
	2	440	80%	216.6	80%
	3	440	80%	216.6	80%
	4	440	80%	216.6	80%
	5	440	80%	216.6	80%
16	1	340	74%	190	79%
	2	340	74%	190	79%
	3	340	74%	190	79%
	4	340	74%	190	79%
	5	340	74%	190	79%
17	1	360	80%	207.5	80%
	2	360	80%	207.5	80%
	3	360	80%	207.5	80%
	4	360	80%	207.5	80%
	5	360	80%	207.5	80%
19	1	360	80%	190	70%
	2	360	80%	175	65%
	3	360	80%	170	63%

	4	360	80%	170	63%
	5	270	60%	170	63%
20	1	240	80%	175	80%
	2	240	80%	175	80%
	3	240	80%	175	80%
	4	240	80%	175	80%
	5	240	80%	175	80%

Table 4: MILK Post-Training Acute Exercise Bout

Subject	Set	Leg Press Acute test (lbs)	Leg Press % of 1 RM	Leg Extension Acute test (lbs)	Leg Extension % of 1 RM
2*	1	375	80%	160	80%
	2	375	80%	160	80%
	3	375	80%	160	80%
	4	375	80%	160	80%
	5	375	80%	160	80%
4**	1	255	80%	235	80%
	2	255	80%	235	80%
	3	255	80%	235	80%
	4	255	80%	235	80%
	5	255	80%	235	80%
6	1	580	79%	255	80%
	2	580	79%	240	75%
	3	580	79%	240	75%
	4	580	79%	240	75%
	5	580	79%	240	75%
7	1	470	80%	227.5	80%
	2	470	80%	227.5	80%
	3	470	80%	227.5	80%
	4	470	80%	227.5	80%
	5	470	80%	227.5	80%
10	1	520	80%	255	80%
	2	520	80%	255	80%
	3	520	80%	255	80%
	4	520	80%	255	80%
	5	520	80%	255	80%
11	1	575	80%	280	86%
	2	575	80%	280	86%
	3	575	80%	280	86%
	4	575	80%	280	86%
	5	575	80%	280	86%
16	1	500	78%	230	75%
	2	500	78%	230	75%
	3	500	78%	230	75%
	4	500	78%	230	75%
	5	500	78%	230	75%
17***	1	592.5	80%	175	95%

	2	592.5	80%	175	95%
	3	592.5	80%	170	92%
	4	592.5	80%	170	92%
	5	592.5	80%	170	92%
19	1	580	79%	295	81%
	2	580	79%	295	81%
	3	580	79%	275	75%
	4	580	79%	255	70%
	5	580	79%	255	70%
20	1	420	79%	240	77%
	2	420	79%	240	77%
	3	420	79%	240	77%
	4	420	79%	240	77%
	5	420	79%	240	77%

*denotes use of 1 leg for leg press and leg extension
**denotes use of one leg for leg press
***denotes use of one leg for leg extension

Table 5: URINE VOLUME

Subject	Pre-Exercise Day 1	Pre-Exercise Day 2	Post-Exercise Day 1	Post-Exercise Day 2
1	1560 mL	880 mL	2360 mL	560 mL
2	1520 mL	1450 mL	640 mL	1330 mL
3	1200 mL	2970 mL	2320 mL	550 mL
4	1400 mL	880 mL	655 mL	810 mL
5	No sample	No sample	1900 mL	880 mL
6	1000 mL	1530 mL	No sample	2370 mL
7	600 mL	700 mL	675 mL	360 mL
10	810 mL	1260 mL	1270 mL	200 mL
11	1370 mL	670 mL	No sample	1450 mL
12	940 mL	1220 mL	830 mL	1950 mL
13	1120 mL	1280 mL	800 mL	1520 mL
14	400 mL	1960 mL	825 mL	710 mL
15	2020 mL	990 mL	1410 mL	1220 mL
16	2140 mL	1100 mL	2660 mL	2410 mL
17	1280 mL	2890 mL	1520 mL	1860 mL
18	600 mL	No sample	990 mL	560 mL
19	490 mL	630 mL	1120 mL	1010 mL
20	580 mL	1070 mL	620 mL	1340 mL
21	840 mL	1580 mL	1500 mL	2520 mL

Table 6: CREATININE EXCRETION (g/24 h)

CHO	CHO Pre-Train Day 1	CHO Pre-Train Day 2	CHO Post-Train Day 1	CHO Post-Train Day 2	MILK	MILK Pre-Train Day 1	MILK Pre-Train Day 2	MILK Post-Train Day 1	MILK Post-Train Day 2
1	2.292	1.358	2.256	0.897	2	2.184	2.653	1.878	2.837
3	1.201	3.647	2.731	0.557	4	1.373	0.813	1.642	0.994
5			4.304	0.944	6	1.425	0.913		2.292
12	1.591	1.098	1.477	3.515	7	1.619	1.596	1.479	1.032
13	1.540	1.655	0.989	0.879	10	1.967	3.423	1.798	
14	1.063	2.485	1.332	1.533	11	2.225	0.931		1.604
15	1.620	0.745	1.286	0.925	16	2.362	1.594	0.412	0.264
18	1.520		2.832	2.391	17	2.357	2.032	2.403	1.349
21	0.670	0.776	1.196	0.696	19	1.545	1.046	2.122	1.599
					20	0.787	2.595	1.179	1.163
Mean	**1.437**	**1.681**	**2.045**	**1.371**	**Mean**	**1.784**	**1.760**	**1.614**	**1.459**
Std Dev	**0.477**	**1.053**	**1.089**	**0.977**	**Std Dev**	**0.519**	**0.894**	**0.614**	**0.755**

Blank spaces in table indicate lack of urine sample for this measurement

Table 7: 3-METHYLHISTIDINE EXCRETION (μmol/day)

CHO	Pre-Training Day 1	Pre-Training Day 2	Post-Training Day 1	Post-Training Day 2	MILK	Pre-Training Day 1	Pre-Training Day 2	Post-Training Day 1	Post-Training Day 2
1	84.93	47.04	73.76	24.47	2	154.08	121.28	52.36	44.03
3	100.59	53.90	52.96	19.27	4	54.79	40.01	23.46	19.80
5			630.97	58.98	6	106.38	42.26		48.88
12	54.37	64.24	43.74	30.08	7	71.16	60.82	57.51	46.22
13	40.13	42.49	31.32		10	33.63	81.56	37.71	
14	66.55	176.65	176.42	126.48	11	232.33	118.56		337.23
15	105.44	28.49	187.00	46.34	16	136.70	57.15		
18	41.96		81.74	30.32	17	131.56	59.28	40.60	
21	33.63		207.62		19	51.77	37.47	280.44	125.24
					20	29.79	44.15	47.87	25.89
Mean	**65.95**	**68.80**	**165.06**	**47.99**		**100.22**	**66.25**	**77.14**	**92.47**
Std Dev	**28.13**	**54.16**	**187.06**	**37.18**		**64.43**	**31.16**	**90.33**	**113.34**

Blank spaces in table indicate lack of urine sample for this measurement, or overfilling of the collection bottle, which caused the bottle to burst, compromising the integrity of the sample.

Table 8: 3-METHYLHISTIDINE-TO-CREATININE RATIO (μmol 3-methylhistidine/g creatinine)

CHO	Pre-Training Day 1	Pre-Training Day 2	Post-Training Day 1	Post-Training Day 2	MILK	Pre-Training Day 1	Pre-Training Day 2	Post-Training Day 1	Post-Training Day 2
1	37.05	34.64	32.70	27.28	2	70.55	45.71	27.88	15.52
3	83.76	14.78	19.39	34.60	4	39.91	49.21	14.29	19.92
5			146.60	62.48	6	74.65	46.29		21.33
12	34.17	58.51	29.61	8.56	7	43.95	38.11	38.88	44.79
13	26.06	25.67	31.67		10	17.10	23.83	20.97	
14	62.61	71.09	132.45	82.50	11	104.42	127.35		210.24
15	65.09	38.24	145.41	50.10	16	57.87	35.85		
18	27.61		28.86	12.68	17	55.82	29.17	16.90	
21	50.19		173.60		19	33.51	35.82	132.16	78.32
					20	37.85	17.01	40.60	22.26
Mean	**48.32**	**40.49**	**82.25**	**39.74**		**53.56**	**44.84**	**41.67**	**58.91**
Std Dev	**20.69**	**20.89**	**64.79**	**26.89**		**24.95**	**30.73**	**41.19**	**70.28**

Blank spaces in table indicate lack of urine sample for this measurement, or overfilling of the collection bottle, which caused the bottle to burst, compromising the integrity of the sample.

Table 9: SERUM GLUCOSE (mg/dL)

CHO	Pre-Train Pre-Ex	Pre-Train Imm Post	Pre-Train 30 min Post	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train Imm Post	Post-Train 30 min Post	Post-Train 1 Hr Post
1	71.84	84.90	128.98	69.80	70.61	64.08	102.86	53.06
3	73.68	70.04	115.39	48.99	67.21	68.02	89.48	50.20
5	67.51	76.37	106.33	52.75	75.95	70.89	120.68	84.39
12	90.16	86.89	103.28	72.14	78.15	75.96	95.08	63.39
13	51.97	60.70	113.54	82.10	60.27	64.63	101.75	55.02
14	72.98	83.79	120.00	69.19	71.35	72.43	116.22	59.46
15	63.44	83.87	124.73	86.02	97.85	93.55	139.78	110.75
18	57.70	55.98	85.47	54.28	48.72	50.43	83.34	50.43
21	65.22	65.22	101.09	49.46	60.33	58.15	111.42	46.20
Mean	**68.28**	**74.20**	**110.98**	**64.97**	**70.05**	**68.68**	**106.73**	**63.66**
Std Dev	**10.92**	**11.61**	**13.46**	**14.09**	**13.82**	**12.11**	**17.39**	**20.96**

MILK	Pre-Train Pre-Ex	Pre-Train Imm Post	Pre-Train 30 min Post	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train Imm Post	Post-Train 30 min Post	Post-Train 1 Hr Post
2	73.45	86.29	108.41	79.65	71.24	67.70	117.26	66.82
4	47.25	62.99	86.61	45.67	65.35	64.57	81.50	49.21
6	62.09	57.92	49.17	51.25	72.09	62.92	94.17	53.33
7	76.58	74.77	74.33	56.76	68.47	75.23	90.09	47.30
10	56.37	57.27	82.73	69.09	56.36	57.73	84.09	43.64
11	66.06	71.50	75.11	81.45	63.35	57.47	63.80	47.06
16	100.54	95.16	108.60	71.51	95.16	87.64	74.27	69.00
17	83.77	71.36	81.55	65.32	69.42	50.49	99.03	41.75
19	72.48	83.95	99.54	62.85	76.15*	77.07*	65.14*	47.95*
20	68.43	61.99	94.16	57.31	88.89	90.65	101.76	31.00
Mean	**70.70**	**72.32**	**86.02**	**64.09**	**72.26**	**68.27**	**89.55**	**49.90**
Std Dev	**14.77**	**12.86**	**17.97**	**11.69**	**12.27**	**13.74**	**15.89**	**11.94**

*indicates data was not used for analysis because Pre-Exercise serum insulin value was not within normal range for overnight fast

Table 10: SERUM INSULIN (μIU/mL)

CHO	Pre-Train Pre-Ex	Pre-Train Imm Post	Pre-Train 30 min Post	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train Imm Post	Post-Train 30 min Post	Post-Train 1 Hr Post
1	13.001	11.283	185.900	103.240	20.701	14.744	107.600	100.500
3	4.957	11.606	130.860	35.701	22.993	13.100	193.420	43.981
5	7.606	9.890	74.723	119.630	14.561	10.990	106.300	96.663
12	9.069	8.129	69.866	56.700	6.798	4.000	40.491	28.834
13	11.907	9.527	84.669	31.117	16.944	6.759	94.486	50.545
14	7.429	4.000	50.596	50.639	9.426	4.241	64.694	21.662
15	5.616	8.007	75.057	61.187	8.366	11.745	58.287	187.560
18	14.725	12.093	61.776	54.468	17.216	5.017	78.275	67.041
21	5.492	5.779	63.082	19.096	5.195	8.650	52.039	87.935
Mean	**8.867**	**8.924**	**88.503**	**59.086**	**13.578**	**8.805**	**88.399**	**76.080**
Std Dev	**3.564**	**2.732**	**43.027**	**32.875**	**6.379**	**4.025**	**46.022**	**50.678**

MILK Group	Pre-Train Pre-Ex	Pre-Train Imm Post	Pre-Train 30 min Post	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train Imm Post	Post-Train 30 min Post	Post-Train 1 Hr Post
2	12.427	12.084	68.671	134.260	32.486	20.030	109.260	85.194
4	6.838	6.088	97.661	28.978	11.160	5.227	49.518	14.715
6	5.919	5.388	37.720	24.085	5.202	7.419	83.421	13.604
7	7.401	6.956	45.991	10.876	8.748	5.373	116.210	16.070
10	7.015	6.536	104.650	31.899	12.845	23.680	117.380	21.508
11	14.101	8.137	68.128	25.835	24.708	4.289	44.891	19.404
16	22.174	12.964	111.600	67.466	23.618	4.000	100.560	19.730
17	11.286	5.457	138.65	42.707	10.383	7.306	86.526	26.388
19	12.670	7.503	127.520	64.965	88.840*	8.088*	76.337*	79.206*
20	9.304	6.390	54.784	12.733	8.371	7.348	64.965	25.927
Mean	**10.914**	**7.750**	**85.538**	**44.380**	**15.280**	**9.408**	**85.859**	**26.949**
Std Dev	**4.878**	**2.661**	**35.212**	**36.985**	**9.309**	**7.231**	**27.660**	**22.301**

*indicates data was not used for analysis because Pre-Exercise value was not within normal range for overnight fast

Table 11: SERUM IGF-1 (μg/L)

CHO	Pre-Train Pre-Ex	Pre-Train Imm Post	Pre-Train 30 min Post	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train Imm Post	Post-Train 30 min Post	Post-Train 1 Hr Post
1	438.77	494.34	416.28	404.32	612.36	490.32	432.01	438.19
3	330.71	398.96	401.20	358.78	528.53	380.34	353.03	251.21
5	403.93	453.46	439.87	438.52	389.93	319.62	323.18	367.99
12	337.77	506.62	365.76	351.78	339.52	380.42	462.99	454.41
13	558.12	475.97	422.33	536.01	522.84	553.06	431.02	360.98
14	387.52	423.79	298.26	347.91	298.68	408.95	323.37	306.89
15	681.26	652.77	498.69	810.52	616.22	918.57	819.01	666.91
18	653.52	516.62	534.08	490.32	523.84	712.21	457.81	442.23
21	245.27	283.82	200.45	169.11	275.56	255.44	173.07	162.30
Mean	**448.54**	**467.37**	**397.44**	**434.14**	**456.39**	**490.99**	**419.50**	**383.46**
Std Dev	**150.61**	**99.75**	**100.84**	**175.44**	**132.14**	**209.66**	**175.68**	**143.54**

MILK	Pre-Train Pre-Ex	Pre-Train Imm Post	Pre-Train 30 min Post	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train Imm Post	Post-Train 30 min Post	Post-Train 1 Hr Post
2	364.18	331.50	338.11	237.64	499.45	381.45	379.45	399.42
4	264.22	252.34	346.94	304.09	312.16	330.90	220.28	325.78
6	225.06	209.96	225.91	261.99	281.65	251.48	279.55	326.30
7	341.96	363.75	444.78	390.31	514.47	482.54	334.36	595.25
10	397.49	327.55	404.87	388.42	458.25	671.14	546.17	663.73
11	349.22	333.76	385.56	351.92	262.71	329.32	219.44	327.15
16	306.27	250.35	284.47	294.49	292.80	333.23	337.17	327.61
17	458.33	474.61	403.54	338.92	340.87	413.32	340.87	317.57
19	239.38	249.97	233.07	208.71	242.42	229.09	320.39	191.76
20	597.17	593.10	407.93	473.35	644.84	640.18	650.16	446.40
Mean	**354.33**	**338.69**	**347.52**	**324.98**	**384.96**	**406.27**	**362.78**	**392.10**
Std Dev	**111.69**	**117.25**	**76.69**	**80.07**	**135.18**	**150.42**	**136.63**	**141.76**

Table 12: SERUM TOTAL AMINO ACIDS (except cysteine) (mg/L)

CHO	Pre-Train Pre-Ex	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train 1 Hr Post	MILK	Pre-Train Pre-Ex	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train 1 Hr Post
1	374.39		355.17	280.23	2	409.04	441.26	346.71	404.08
3	350.16	362.99	387.54	298.12	4	339.48	420.22	364.24	355.47
5	413.92	391.31	452.08	363.76	6	144.78	369.00	335.36	331.74
12	371.55	353.49	353.39	297.10	7	346.24	366.22	384.10	348.03
13	354.32	323.11	398.36	300.90	10	531.83	435.32	409.48	432.61
14	378.10	291.41	307.58	284.13	11	135.02	442.52	329.20	415.46
15	351.35	318.03	319.86	296.21	16	307.96	394.45	381.21	438.92
18	345.09	359.92	312.97	292.74	17	379.96	390.35	352.70	413.38
21	371.30	312.42	380.58	308.75	19	344.83	334.62	473.61	517.05
Mean	**367.8**	**339.09**	**363.06**	**302.44**	20	360.37	413.40	349.47	430.51
Std Dev	**21.16**	**33.00**	**47.02**	**24.52**	**Mean**	**329.95**	**400.74**	**372.61**	**408.73**
					Std Dev	**117.28**	**36.27**	**43.09**	**53.98**

Blank space indicates missing data due to lack of blood sample for this measurement

Table 13: SERUM ESSENTIAL AMINO ACIDS (mg/L)

CHO	Pre-Train Pre-Ex	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train 1 Hr Post	MILK	Pre-Train Pre-Ex	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train 1 Hr Post
1	149.52		137.30	99.99	2	155.54	164.88	134.52	156.17
3	133.43	114.60	138.51	95.09	4	129.26	155.28	130.70	119.52
5	155.05	118.80	163.40	117.07	6	53.30	134.67	120.35	119.72
12	146.43	117.93	134.31	98.72	7	124.60	120.40	136.55	122.02
13	133.24	100.82	157.12	101.29	10	206.24	153.72	153.77	151.62
14	138.27	91.75	124.34	101.16	11	47.31	147.48	111.10	150.98
15	142.44	104.59	123.21	94.77	16	111.26	139.91	134.20	166.91
18	139.39	122.72	132.29	102.73	17	150.07	143.05	132.22	149.02
21	151.87	102.49	135.70	92.69	19	138.18	128.82	188.53	208.49
					20	123.97	143.19	137.99	169.73
Mean	**143.29**	**109.21**	**138.46**	**100.39**	**Mean**	**123.97**	**143.14**	**137.99**	**151.42**
Std Dev	**7.91**	**10.83**	**13.54**	**7.13**	**Std Dev**	**46.80**	**13.15**	**20.96**	**27.38**

Blank space indicates missing data due to lack of blood sample for this measurement

Table 14: SERUM LEUCINE (mg/L)

CHO	Pre-Train Pre-Ex	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train 1 Hr Post	MILK	Pre-Train Pre-Ex	Pre-Train 1 Hr Post	Post-Train Pre-Ex	Post-Train 1 Hr Post
1	23.69		21.66	14.21	2	24.45	26.41	20.82	24.27
3	19.44	12.09	22.51	13.21	4	18.65	22.22	19.93	17.46
5	22.94	14.22	25.93	16.34	6	7.19	17.82	18.66	16.03
12	21.32	14.39	20.78	12.31	7	17.35	15.08	22.28	17.45
13	19.36	10.95	22.58	11.89	10	32.08	21.21	24.16	22.60
14	22.20	12.31	18.69	12.80	11	7.44	20.81	16.75	25.36
15	22.91	10.54	19.56	11.90	16	16.50	20.66	18.02	26.27
18	22.58	15.25	23.31	12.90	17	22.16	20.81	20.06	22.42
21	24.83	11.53	21.73	12.41	19	20.43	19.33	30.59	34.40
Mean	**22.14**	**12.66**	**21.86**	**13.11**	20	17.15	19.90	20.55	23.62
Std Dev	**1.83**	**1.74**	**2.13**	**1.41**	**Mean**	**18.34**	**20.43**	**21.18**	**22.99**
					Std Dev	**7.41**	**2.92**	**3.92**	**5.36**

Blank space indicates missing data due to lack of blood sample for this measurement

Likert Scale Results for Post-Exercise Beverage

Distance from the Left End of Scale (cm)

15 cm scale ranges from "not at all" on far left to "completely" on far right

Table 15: TASTES GOOD

CHO

	Pre-Training		Post-Training	
ID	**Immediate Post**	**1 Hour Post**	**Immediate Post**	**1 Hour Post**
1	10.3	10.9	11.4	11.0
3	6.8	7.3	4.2	4.1
5	0	0	0	0
12	6.5	6.0	7.7	7.6
13	4.0	3.2	10.8	11.6
14	11.1	10.2	12.5	13.4
15	7.7	4.6	5.7	8.7
18	7.0	6.4	5.4	6.5
21	10.2	10.5	10.5	9.1
Mean	**7.1**	**6.6**	**7.6**	**8.0**
SD	**3.5**	**3.7**	**4.1**	**4.1**
SEM	**1.2**	**1.2**	**1.4**	**1.4**

MILK

	Pre-Training		Post-Training	
ID	**Immediate Post**	**1 Hour Post**	**Immediate Post**	**1 Hour Post**
2	15.0	15.0	10.6	11.1
4	13.5	13.4	13.5	13.3
6	6.2	4.9	12.2	11.6
7	8.5	4.8	7.2	8.0
10	11.0	9.4	11.9	12.7
11	9.7	10.3	12.3	13.6
16	11.3	11.5	10.8	10.3
17	11.9	12.0	10.4	10.6
19	10.7	10.4	10.6	10.4
20	12.8	12.0	13.1	13.4
Mean	**11.1**	**10.4**	**11.3**	**11.5**
SD	**2.5**	**3.3**	**1.8**	**1.8**
SEM	**0.8**	**1.0**	**0.6**	**0.6**

Table 16: QUENCHES THIRST

CHO

	Pre-Training		Post-Training	
ID	**Immediate Post**	**1 Hour Post**	**Immediate Post**	**1 Hour Post**
1	9.9	11.7	11.4	11.1
3	11.3	10.9	5.4	4.9
5	4.2	2.9	7.5	7.1
12	5.8	6.5	7.6	7.2
13	8.7	12.0	13.0	8.7
14	11.0	10.0	12.0	13.7
15	5.3	7.8	6.8	10.0
18	8.7	8.5	8.6	7.9
21	10.4	10.2	7.2	7.7
Mean	**8.4**	**8.9**	**8.8**	**8.7**
SD	**2.6**	**2.9**	**2.6**	**2.6**
SEM	**0.9**	**1.0**	**0.9**	**0.9**

MILK

	Pre-Training		Post-Training	
ID	**Immediate Post**	**1 Hour Post**	**Immediate Post**	**1 Hour Post**
2	3.0	5.6	3.1	4.6
4	9.8	13.0	10.7	11.9
6	4.6	3.4	9.2	10.5
7	5.5	4.8	11.5	5.2
10	11.9	8.6	12.0	10.2
11	9.6	10.3	11.4	12.3
16	4.5	11.2	3.3	1.1
17	8.5	9.8	7.2	6.2
19	5.4	6.0	5.4	4.2
20	3.8	5.2	9.6	11.4
Mean	**6.7**	**7.8**	**8.3**	**7.8**
SD	**3.0**	**3.2**	**3.4**	**4.0**
SEM	**1.0**	**1.0**	**1.1**	**1.3**

Table 17: FULFILLS HUNGER

CHO

	Pre-Training		Post-Training	
ID	**Immediate Post**	**1 Hour Post**	**Immediate Post**	**1 Hour Post**
1	13.5	13.5	6.5	7.3
3	9.4	9.2	6.9	5.1
5	0	5.6	8.5	7.3
12	6.6	9.3	7.6	7.2
13	5.5	8.5	8.5	10.6
14	7.7	10.4	6.3	13.8
15	13.9	5.6	10.0	11.8
18	5.9	6.6	7.4	7.5
21	4.9	10.0	4.7	7.7
Mean	**7.5**	**8.7**	**7.4**	**8.7**
SD	**4.3**	**2.5**	**1.5**	**2.8**
SEM	**1.4**	**0.8**	**0.5**	**0.9**

MILK

	Pre-Training		Post-Training	
ID	**Immediate Post**	**1 Hour Post**	**Immediate Post**	**1 Hour Post**
2	5.0	5.3	5.6	4.7
4	11.6	10.6	12.0	13.0
6	0	0	4.3	5.0
7	4.8	4.2	11.2	4.9
10	9.3	9.4	12.8	10.6
11	7.5	8.3	9.5	8.0
16	15.0	15.0	7.4	10.4
17	7.1	7.0	9.8	6.3
19	10.1	9.7	9.2	9.6
20	9.9	13.0	11.4	11.8
Mean	**8.0**	**8.3**	**9.3**	**8.4**
SD	**4.2**	**4.4**	**2.8**	**3.1**
SEM	**1.3**	**1.4**	**0.9**	**1.0**

Table 18: ENERGIZING

CHO

	Pre-Training		Post-Training	
ID	**Immediate Post**	**1 Hour Post**	**Immediate Post**	**1 Hour Post**
1	10.8	11.0	8.0	9.1
3	10.0	7.5	7.9	7.5
5	5.0	3.2	0	0
12	6.0	8.3	7.7	7.4
13	10.5	12.5	11.2	10.8
14	8.3	10.6	6.2	8.7
15	9.4	8.2	9.1	7.6
18	6.4	7.0	7.6	6.5
21	7.6	7.2	7.1	8.9
Mean	**8.2**	**8.4**	**7.2**	**7.4**
SD	**2.1**	**2.7**	**3.0**	**3.0**
SEM	**0.7**	**0.9**	**1.0**	**1.0**

MILK

	Pre-Training		Post-Training	
ID	**Immediate Post**	**1 Hour Post**	**Immediate Post**	**1 Hour Post**
2	0	0	0	0
4	7.3	10.6	10.8	12.1
6	3.9	7.8	9.2	9.1
7	5.1	5.0	4.7	5.6
10	11.6	11.7	10.7	12.6
11	7.8	6.6	8.1	8.2
16	7.5	0	1.0	15.0
17	5.5	4.8	3.9	6.2
19	6.1	6.2	4.1	5.7
20	6.7	6.3	11.5	11.8
Mean	**6.2**	**5.9**	**6.4**	**8.6**
SD	**3.0**	**3.8**	**4.2**	**4.4**
SEM	**0.9**	**1.2**	**1.3**	**1.4**

Appendix C: Statistical Analyses of Measurements

Statistical Procedures

The differences between groups for Likert scale results were analyzed by one-way ANOVA. Measurements of blood metabolites and hormones, and urinary measures that were done before and after the two acute resistance tests were analyzed with three-way repeated measures ANOVA (group, training status, pre/post resistance exercise). A mixed model technique was applied to account for two layers of correlation: 1) same subject used for multiple measurements and 2) multiple measurements of the same variable made in a short time period are correlated. A p value less than 0.05 was considered significant.

Table 19: ANOVA for Serum Essential Amino Acids

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	30.1	4.17	0.0500
period	1	17.2	0.02	0.9027
Time	1	36.3	5.71	0.0222
GROUP*time	1	36.3	39.76	<0.0001

Num = Numerator, Den = Denominator (Error)

Table 20: ANOVA for Serum Leucine

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	22.8	5.47	0.0285
Period	1	17.6	2.84	0.1095
Time	1	36.2	25.63	<0.0001
GROUP*time	1	36.2	61.03	<0.0001

Num = Numerator, Den = Denominator (Error)

Table 21: ANOVA for Serum Total Amino Acids

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	27.9	4.34	0.0465
period	1	19.8	0.43	0.5208
time	1	36.3	0.13	0.7222
GROUP*time	1	36.3	20.06	<0.0001

Num = Numerator, Den = Denominator (Error)

Table 22: ANOVA for Serum IGF-1

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	16.7	5.25	0.0352
period	1	18	2.15	0.1596
time	3	77	3.56	0.0179

Num = Numerator, Den = Denominator (Error)

Table 23: ANOVA for Serum Glucose

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	17.2	1.99	0.1765
period	1	17.3	1.11	0.3056
time	3	69.6	110.05	<0.0001
GROUP*time	3	69.6	9.36	<0.0001

Num = Numerator, Den = Denominator (Error)

Table 24: ANOVA for Serum Insulin

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	16.8	1.50	0.2380
period	1	17.2	0.27	0.6088
time	3	76.4	79.14	<0.0001
GROUP*time	3	76.4	3.52	0.0190

Num = Numerator, Den = Denominator (Error)

Table 25: ANOVA for Creatinine Excretion

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	13.6	0.26	0.6216
period	1	13.2	0.02	0.8929
time	1	35.8	0.62	0.4359

Num = Numerator, Den = Denominator (Error)

Table 26: ANOVA for 3-Methylhistidine Excretion

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	17.2	0.00	0.9649
period	1	15.9	0.23	0.6389
time	1	34.1	4.54	0.404

Num = Numerator, Den = Denominator (Error)

Table 27: ANOVA for 3-Methylhistidine-to-Creatinine ratio

Effect	Num DF	Den DF	F Value	Pr>F
GROUP	1	14.4	0.13	0.7225
period	1	16.8	1.17	0.2954
time	1	29.9	10.94	0.0025
period*time	1	30.2	3.97	0.0555

Num = Numerator, Den = Denominator (Error)

Table 28: One-Way ANOVA for Taste of Beverage Pre-Training

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75.537	1	75.537	8.333	.010
Within Groups	154.104	17	9.065		
Total	229.641	18			

Table 29: One-Way ANOVA for Taste of Beverage Post-Training

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	64.226	1	64.226	6.659	.019
Within Groups	163.960	17	9.645		
Total	228.185	18			

Table 30: One-Way ANOVA for Quenching of Thirst by Beverage Pre-Training

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.797	1	13.797	1.695	.210
Within Groups	138.364	17	8.139		
Total	152.161	18			

Table 31: One-Way ANOVA for Quenching of Thirst by Beverage Post-Training

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.153	1	1.153	.123	.730
Within Groups	159.364	17	9.374		
Total	160.517	18			

Table 32: One-Way ANOVA for Fulfillment of Hunger by Beverage Pre-Training

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.387	1	1.387	.077	.785
Within Groups	306.950	17	18.056		
Total	308.337	18			

Table 33: One-Way ANOVA for Fulfillment of Hunger by Beverage Post-Training

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.868	1	17.868	3.416	.082
Within Groups	88.932	17	5.231		
Total	106.800	18			

Table 34: One-Way ANOVA for Energizing by Beverage Pre-Training

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.340	1	20.340	2.989	.102
Within Groups	115.701	17	6.806		
Total	136.041	18			

Table 35: One-Way ANOVA for Energizing by Beverage Post-Training

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.032	1	3.032	.221	.644
Within Groups	233.140	17	13.714		
Total	236.172	18			

Appendix D: Health History Questionnaire

SUPPLEMENTARY QUESTIONS

Food Habits and Allergies

1. Are you allergic to any foods? ____ If yes, which ones?

2. Are you on any kind of special diet? ____ If so, what kind?

1. Do you take any dietary supplements?_____ If so, what kind and how often?

2. Has your weight been stable over the past year?____ If not, how has it changed?

Drug Allergies

3. Are you aware of any allergies you have to any drugs? ____ If yes, which ones?

4. Have you ever received Novocaine at the dentist's office or other local (injected into skin) anesthetic? _____.

If yes, did you have any allergic reaction to this?_____

Comfort with procedures

5. Do you have a fear of needles or having blood withdrawn?

8. Is there anything about the muscle sampling procedures that particularly concerns you? Explain.

VIRGINIA TECH LABORATORY FOR HEALTH AND EXERCISE SCIENCE
MEDICAL AND HEALTH HISTORY

Name: _____ Age: _____ Birth Date: _____

Address: _____ e-mail: _____

Phone Numbers: Home: _____ Work :_____

Summer Address: _____
_____

Phone Number (during Winter Break): _____

Person to Contact in Case of an Emergency: _____

Relationship: _____Phone: _____

Primary Care Physician: _____Phone: _____

Medical Insurance Carrier: _____

Are you employed by Virginia Tech? _____

Current Body Weight:_____

MEDICAL HISTORY

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

	Yes	**No**
Heart disease or any heart problems:	_____	_____
Rheumatic Fever:	_____	_____
Respiratory disease or breathing problems (e.g. asthma):	_____	_____
Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Urinary problems:	_____	_____
Musculoskeletal problems:	_____	_____
(i.e. Orthopedic injuries, osteoporosis)		
Fainting and Dizziness:	_____	_____
High Cholesterol:	_____	_____
Diabetes:	_____	_____
Thyroid problems:	_____	_____
Mental illness:	_____	_____
Hypoglycemia:(i.e. low blood sugar)	_____	_____
Epilepsy or seizures:	_____	_____
Blood clotting problems (e.g. hemophilia):	_____	_____

Liver disorders (e.g. hepatitis B) _____ _____

If you answered "yes" to any of the previous questions, please indicate the date and describe:
_____
_____
_____
_____

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

	Yes	No
Have you ever been diagnosed as having high blood pressure?	_____	_____
Are you currently being treated for high blood pressure?	_____	_____

If "yes", please explain:
_____
_____
_____

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

For what reason(s) are you taking this medication?
_____
_____
_____

Health Habits

	Yes	No
Do you drink alcoholic beverages?	_____	_____
How many drinks per week? _____		
Do you smoke cigarettes?	_____	_____
Packs per day: _____		

	Yes	No
Do you engage in regular exercise?	_____	_____

If "yes", please list:

Activity	Frequency (times per week)	Duration (minutes)

_____ _____ _____
_____ _____ _____
_____ _____ _____

Do you ever faint, experience shortness of breath or chest discomfort with exertion? _____

If "yes", please explain: _____
_____

Are there any orthopedic limitations you have that may restrict your ability to perform exercise and if "yes", please explain:
_____
_____
_____

Family History

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

	Yes	**No**	**Relationship**	**Age**
Heart attack	____	____	_____	____
Heart disease	____	____	_____	____
High blood pressure	____	____	_____	____
Stroke	____	____	_____	____
Kidney disease	____	____	_____	____
Diabetes	____	____	_____	____

Schedule Spring 2001 semester (indicate those times you have classes, work etc that you CANNOT be involved in testing or exercise training):

	Mon	*Tue*	*Wed*	*Thursday*	*Fri*
6:00-7:00am					
7:00-8:00					
8:00-9:00					
9:00-10:00					
10:00-11:00					
11:00-12:00					
12:00-1:00					
1:00-2:00					
2:00-3:00					
3:00-4:00					
4:00-5:00					
5:00-6:00					
6:00-7:00					
7:00-8:00					

Any explanation required for above _____
_____

_____

Please sign to indicate that the above information is correct:

_____	_____	_____
Print name	Signature	Date

Appendix E: Institutional Review Board Request

Informed Consent for Participants of Investigative Projects
Department of Human Nutrition, Foods, and Exercise
Virginia Tech

TITLE: The Role of Recovery Beverages on Training Adaptations to Resistance Exercise.

PHASE II.

PRINCIPAL INVESTIGATOR: Janet Walberg Rankin, Ph.D., graduate students: Lauren Goldman and Michael Puglisi

PURPOSE:

Some people consume special beverages after resistance exercise to enhance recovery after the workout. This study will examine the effect of several different beverages on muscle protein synthesis and breakdown that results from consumption of these drinks after a strenuous resistance training workout. In addition, we will determine whether individuals get more muscle mass or strength gains with one supplement over the other when they participate in regular resistance weight training.

General Design

The study will last approximately 12 weeks with the first week being baseline testing of strength, diet and body composition. The next 10 weeks will include a resistance training program 3 days/wk. We will give you a beverage to consume after each workout..

Once at the first week of the training and once during the last week of training, we will ask you to come to our laboratory in the morning without eating breakfast. We will take a small sample of your muscle as well as a blood sample while you are resting. Then, we will ask you to do a strenuous resistance training workout designed for you based on your muscle strength. It will include 5 sets of 8 repetitions at 80% of your maximal strength of leg extension and leg press. We will give you a beverage to consume as soon as you are done. We will take another sample of your muscle and blood at 1 hour after you complete the workout.

Body Composition: We will use two methods to estimate your body composition (body fat and lean tissue). One method uses a machine called DEXA to measure your body fat and lean body mass. This involves lying still on a flat bed for about 15 minutes while a beam passes over your body. This procedure uses 2 low energy X rays to determine your body fat. You will not feel anything during the procedure but need to remain very still. The second method for body composition estimation is bioelectric impedance. Measurements will be taken by three different devices, one after the other. You will remove your right sock and shoe, all jewelry and any metal objects, then you will have your hand and foot gently cleaned with an alcohol pad, after which four small wires will be attached to your hand and foot by a sticky pad called an electrode. A small, undetectable electrical signal will be sent through these wires through your body by the bioelectrical impedance analyzer. The resistance of your body to this signal will provide an estimate of body water, lean mass, and percent body fat. The measurement by each device will last less than 10 seconds in duration, and measurement by all three devices will take approximately 5 - 10 minutes.

1 Repetition Maximum (1RM)-

Prior to the start of the strength training program and at the end of the program, we will test your maximal strength for eight lifts. This will involve warming up with a weight you think you can lift about 10 times, resting, then progressively trying heavier weights until you can only lift the weight one time.

Muscle Samples
Since aspirin reduces blood clotting, you should not take aspirin for 24 h prior to having this procedure. A small sample of your thigh muscle will be taken just above the knee and to the outside of your leg. The area will be shaved and cleaned. A local anesthetic will be injected to the area (will feel like bee sting) to numb it. A half inch incision will be made with a scalpel after which a hollow needle will be inserted into the incision. A small piece of muscle will be removed with the needle (less than half the size of a pencil eraser). Some people feel nothing at all while others feel cramping or pressure when the sample is removed. After the needle is removed, we will apply pressure and then cold to the incision for about 20 minutes. The incision will be closed with a steri-strip (similar to a band-aid) and will be covered with a pressure wrap. The pressure bandage should be left on for about 8 hours and the steri-strips should remain on for about 3 days. You should not "baby" the leg; using it will prevent excessive stiffness. The incision may be sore for a few days as it heals. It is important to keep the area clean. You may take over the counter pain medication after having the muscle sample procedure if you feel that it is necessary. You will be provided with a written handout containing instructions for treatment of the biopsy incision as well as contact phone numbers for the physician involved in the study and the principal investigator. We want you to call both these individuals immediately if you have a concern about the biopsy incision or experience any adverse effects from the procedure. The incision will close and begin healing within a few days but a small scar will remain. You will have a total of 4 scars (2 on each thigh) at the end of the study. We will show you a photo of a scar from a former subject. The muscle sample we remove will be frozen and later analyzed for indicators of muscle protein synthesis. The biopsy procedure lasts about 30 minutes.

The biopsy procedure will be performed by a Certified Medical Laboratory Technician experienced in the procedure. In addition, a physician will be available in case of emergency. We have not had anyone who required any medical attention following muscle biopsies in the past. Over 200 muscle biopsies have been performed in previous studies.

Blood Samples
You will have three blood samples taken on the day of the resistance exercise test at the beginning and again at the end of the training program. The amount in each sample is about 2 teaspoons.

Urine Collections
You will be asked to collect all the urine you produce for a total of 4 days (2 days at the beginning and 2 days at the end) throughout the experiment. We will provide you with plastic containers to use to collect and store the urine over the day. You should bring it in to us in the morning and we will provide you with new bottles. We will measure a factor in the urine that indicates muscle protein breakdown.

SUBJECT RESPONSIBILITIES

1. Participate in the regular resistance training sessions 3 times per week for 10 weeks with a partner at the Virginia Tech Recreation Center.
2. Consume the provided beverage after each workout session.

3. Refrain from taking any other nutritional supplements without checking first with the experimenters.
4. Give maximal effort on performance tests.
5. Come to the resistance training test in a fasted condition (nothing to eat since the evening before) without having consumed any alcoholic or caffeinated beverages the night before or that morning.
6. Inform the experimenters if you experience any unusual symptom from any of the testing or training.
7. Inform the researchers of any known medical conditions or allergies you are aware of prior to the study as well as any transmittable diseases acquired during the study.
8. Refrain from taking aspirin for 24 hours prior to the muscle sampling procedures (to reduce chance of excess bleeding during the procedures).
9. You must remain in the laboratory for at least 20 minutes after the muscle sampling.
10. Come to the laboratory for the two days after your muscle biopsies so that we can insure they are healing correctly.

RISKS OF PARTICIPATION

1. Fatigue, muscle soreness, muscle strains or pulls may result from the resistance exercise. We will show you proper form to reduce the chance of serious injury.
2. Infection, bruising, muscle soreness from the blood and muscle sampling. The procedures will be conducted by an experienced technician. Universal precautions will be taken such as use of gloves when handling tissue samples. You blood will be screened for HIV if there is accidental exposure of an experimenter with your blood or muscle.
3. An allergic reaction is possible to the injection of local anesthetic prior to the muscle sampling. It is important to tell us if you have ever had an allergic reaction to novocaine or any other anesthetic.
4. The University will not be responsible for any medical expenses you may have unless the University has been negligent.
5. The amount of radiation exposure from the DEXA scan is very low and is about $1/1000^{th}$ of the normal radiation exposure you receive due to normal background radiation from the environment over a year. The exposure is much less than for most X ray tests you would receive at the hospital; for example it is about $1/20^{th}$ of the exposure received during a normal chest X ray. Increased exposure to radiation is associated with increased risk of tumors and cancer. However, the amount of radiation exposure from this scan is less than that acquired during a cross country flight. The amount of radiation in the scan is not expected to significantly increase your risk of cancer but this can not be quantified.

BENEFITS OF PARTICIPATION

Your participation will provide you with:

1. Data on your body composition and muscle strength.
2. A free supervised resistance training program individually designed for you.

COMPENSATION

A total of $200 for full completion of the study. If you complete less than the total study, your payment will be partial; $20 for completion of the baseline testing, $5 for each week of resistance training.

ANOYNMITY AND CONFIDENTIALITY

The data from this study will be kept strictly confidential. No data will be released to anyone but those working on the project without your written permission. Data will be identified

by subject numbers, without anything to identify subjects by name.

FREEDOM TO WITHDRAW

You are free to withdraw at any time from the study for any reason. Circumstances may come up that the researcher will determine that you should not continue as a subject in the study. For example, lack of compliance to diet or exercise, failure to attend testing sessions and illness could be reasons to have the researchers stop your participation in the study.

APPROVAL OF RESEARCH

This research has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech, and by the Department of Human Nutrition, Foods, and Exercise. You will receive a copy of this form to take with you.

SUBJECT PERMISSION

I have read the informed consent and fully understand the procedures and conditions of the project. I have had all my questions answered, and I hereby give my voluntary consent to be a participant in this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If I have questions, I will contact:

- Principal Investigator: Janet Walberg Rankin, Professor, Department of Human Nutrition, Foods, and Exercise. 231-6355
- Chairman, Institutional Review Board for Research Involving Human Subjects:David Moore, 231-4991

Name of Subject (please print)_____

Appendix F: Instructions for Subjects

Guidelines for 1 RM Testing

1. Instruct subject to warm up with light resistance that allows 10 repetitions.

2. Allow 1-minute rest period.

3. Estimate warm up load that will allow subject to complete 3-5 repetitions.

-add 10-20 lbs. for upper body
-add 30-40 lbs. add for lower body

4. Allow 2-minute rest period.

5. Estimate conservative, near maximum load, that will allow subject to complete 2-3 repetitions.

-add 10-20 lbs. for upper body
-add 30-40 lbs. for lower body

6. Allow 2-minute rest period.

7. Increase load so subject will attempt 1-RM.

-add 10-20 lbs. for upper body
-add 30-40 lbs. for lower body

8. If subject can complete successfully, allow 2-minute rest period and repeat step 7.

9. If subject cannot complete successfully, allow 2-minute rest period and decrease load until 1 RM is achieved.

-5-10 lbs. decrease for upper body
-15-20 lbs. decrease for lower body

Resistance Test Instructions

Report to War Memorial Hall at 8 AM on the morning of your resistance test. The test will take approximately 2.5 hours, but plan to be there until 11.

Urine Collections: ***day before test, day of test***
You will be asked to collect all the urine you produce for a total of 4 days (2 days at the beginning and 2 days at the end) throughout the experiment. We will provide you with plastic containers to use to collect and store the urine over the day. You should bring it in to us in the morning and we will provide you with new bottles. We will measure a factor in the urine that indicates muscle protein breakdown. We will set up a time and place to pick up the urine bottles.

Come to the resistance training test in a fasted condition (nothing to eat since the evening before) without having consumed any alcoholic or caffeinated beverages the night before or that morning. Do not eat meat for two days before the test, and on the day of the test. We will provide food for you to consume for the day before and the day of the test. You must mark what you eat and what you waste, and you must not consume foods that we did not provide. Bring this form of your diet to the test. Wear shorts so that the muscle biopsy can be done. Inform the researchers of any known medical conditions or allergies you are aware of prior to the study as well as any transmittable diseases acquired during the study. Refrain from taking aspirin for 24 hours prior to the muscle sampling procedures (to reduce chance of excess bleeding during the procedures). You must remain in the laboratory for at least 20 minutes after the muscle sampling. Come to the laboratory for the two days after your muscle biopsies so that we can insure they are healing correctly.

Instruction for Urine Collection

Urine Collection

Urine analysis will be performed on all urine voided the day of testing as well as one day prior (baseline measure). All urine is to be collected and turned in to the investigators during this time. There will be two 24-hour urine collection periods.

The first collection period ***does not include*** the first voiding on the day before testing, but will begin ***immediately after*** the first voiding. The final collection period will end after the first voiding the morning after testing.

Each collection period begins after the first voiding in the morning and includes the first voiding of the next morning, then a new collection period begins.

You will be given two to three 1-liter containers each day. There will be a small amount of hydrochloric acid in each bottle as a preservative. This is acid, so avoid skin contact. All urine should be voided directly into the collection bottle. Fill one collection bottle before using another bottle. Once a bottle is full, please refrigerate until the bottle can be returned.

Collection bottles are to be turned in at _____ in the morning when you come for testing and in the morning the day after testing.

Instructions for Care of the Muscle Biopsy Area

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

General Instructions

- Keep the pressure wrap on for 8 hours following the biopsy
- Keep the steri-strips and Band-Aid on for 3 days.
- It is OK to shower, but you will want to avoid taking a bath or using a hot tub or swimming pool. Change Band-Aids after showering.
- You are encouraged to walk on that leg; there is no need to "baby" it

Do Not:

- Perform *vigorous* exercise for 2-3 days.
- Get in a river, lake, pool, or hot tub.
- Consume any pain-relief medications without checking with us first.

Normal Reactions Following Muscle Biopsy

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

Reactions Not Normal Following Muscle Biopsy

- Intense, excruciating pain in the leg or in the area of the biopsy
- Bleeding which does not stop
- Intense redness in the area of the biopsy
- Heat in the area of the biopsy
- Presence of pus
- Fever
- Hives or other signs consistent with allergic reaction (ex., difficulty breathing)
- IF YOU EXPERIENCE ANY OF THE ABOVE, CONTACT US IMMEDIATELY REGARDLESS OF THE TIME. IN CASE OF EMERGENCY, DO NOT HESITATE TO GO TO THE EMERGENCY ROOM OR CALL 911

Michael Puglisi: 961-5922, 231-7708
Randy Bird: 953-2610, 231-7708
Dr. Janet Rankin, PhD: 231-6355, 552-9017
Dr. William Epstein, MD: 951-7407
Ms. Janet Rinehart: 231-2667
University Student Health Services: 231-6444

MENU AND INSTRUCTIONS FOR MEAT-FREE DIET

DAY 1

Breakfast
-Omelet
Instructions: 1) Beat eggs with a fork, 2) Add margarine to a frying pan on stove at low heat, 3) Add eggs, 4) Fold in 2 oz cheddar cheese
-Bagel with low fat cream cheese
-Orange juice

Lunch
-Peanut butter and jelly sandwich (whole wheat bread, reduced fat peanut butter, jelly)
-Unsalted twist pretzels
-Banana

Dinner
-Spaghetti (linguini, spaghetti sauce)
Instructions: 1) Bring a quart of water to a rapid boil, 2) Add linguini, 3) Return water to a rapid boil, 4) Cook uncovered stirring frequently for 7-8 minutes or until it reaches the desired tenderness, 5) Drain linguini, 6) Heat sauce in pan on stove
-Dinner roll with margarine
-Tossed salad with low calorie Italian dressing

Snack or Dessert
-Chips Ahoy cookies
-Skim milk

DAY 2

Breakfast
-Raisin Bran cereal with skim milk
-Bagel with reduced fat peanut butter
-Orange juice

Lunch
-Grilled cheese or Veggie burger
Instructions
-Unsalted twist pretzels
-Apple

Dinner
-bean burrito
Instructions

-1 cup of Rice
Instructions: 1) Bring 1 cup of water to a boil, 2) Stir in rice, cover, 3) Remove from heat, 4) Let stand for 5 minutes or until the water is absorbed, 5) Fluff with a fork
Microwave Instructions: 1) Mix 1 cup of water and rice in a bowl, 2) Cover bowl, 3) Cook on high for 6 minutes, 4) Let stand for 5 minutes or until the water is absorbed, 5) Fluff with a fork
-Yellow corn
Instructions: 1) Place ¾ cup of water and corn in pan and bring to a full boil, 2) reduce to a simmer and cook for 12-14 minutes or until tender, 3) drain
Microwave Instructions: 1) Place corn and 2-4 tbsp water in a covered bowl, 2) Cook on high for 9-11 minutes

Snack or Dessert
-Low fat yogurt
-2 tbsp Peanuts

DAY 3

Breakfast
-Raisin Bran cereal with skim milk
-Bagel with low fat cream cheese
-Orange juice

Lunch
-Macaroni and Cheese
Instructions: 1) Place macaroni and 1½ cups of water in a bowl, 2) Microwave on high for 8-10 minutes, stirring every 3 minutes, 3) Add ¼ cup margarine, ¼ cup milk, and cheese sauce, 4) Mix well
-Banana

Dinner
-Baked potato with sour cream, margarine, and cheddar cheese
Instructions: 1) Poke holes with fork, 2) Wrap in foil, 3) Cook at 350° F for 60-70 minutes
Microwave Instructions: 1) Poke holes with fork, 2) Cook for 10 minutes on Medium High
-Broccoli spears
Instructions: 1) Place ¾ cup of water and broccoli in a saucepan, 2) Cover and bring to a full boil, 3) Reduce heat and simmer for 5-7 minutes or until tender, 4) Drain
Microwave Instructions: 1) Place broccoli and 2 tbsp of water in a covered dish, 2) Microwave on high for 6-8 minutes
-Dinner roll with margarine
-Veggie Burger (see instructions on Day 2)

Snack or dessert
-Low fat yogurt
-2 tbsp Peanuts

DAY 4

Breakfast
-Omelet with eggs, 2 oz cheddar cheese and margarine
-Bagel with low fat cream cheese
-Orange juice

Lunch
-Peanut butter and jelly sandwich (whole wheat bread, reduced fat peanut butter, jelly)
-Unsalted twist pretzels
-Apple

Dinner
-Tossed salad with low calorie Italian dressing
-Spaghetti with sauce (linguini and spaghetti sauce; see Instructions on Day 1)
-Dinner roll with margarine

Snack or dessert
-Chips Ahoy cookies
-Skim milk

Appendix G: Example Food Checklist

Subject #6-Food Checklist

Day 1

Breakfast

whole eggs (omelet)	2 items_____
margarine (for cooking omelet)	1 tbsp_____
shredded cheddar cheese (for omelet)	2 oz_____
plain bagel	1 item_____
low fat cream cheese (for bagel)	2 tbsp_____
orange juice	2 cups_____

Lunch

whole wheat bread (for sandwich)	2 slices_____
reduced fat peanut butter (for sandwich)	2 tbsp_____
jelly (for sandwich)	2 tbsp_____
unsalted twist pretzels	10 items_____
banana	1 item_____

Dinner

linguini	2 cups (cooked)_____
spaghetti sauce	1 cup_____
dinner roll	1 item_____
margarine	1 tsp_____
tossed salad	1 cup_____
low calorie Italian dressing	3 tbsp_____

Snacks/Other

Chips Ahoy cookies	2 items_____
skim milk	1 cup_____

Subject #6-Food Checklist

Day 2

Breakfast	
Raisin Bran cereal	2 cups_____
skim milk	1 cup_____
plain bagel	1 item_____
reduced fat peanut butter (for bagel)	2 tbsp_____
orange juice	2 cups_____
Lunch	
Harvest Burgers	2 items_____
hamburger buns	2 items_____
apple	1 item_____
Dinner	
bean burrito	1 item_____
rice	1 cup_____
yellow corn	1.5 cups_____
Snacks/Other	
low fat yogurt	1 cup_____
peanuts	2 tbsp_____

Subject #6-Food Checklist

Day 3

Breakfast	
Raisin Bran cereal	2 cups_____
skim milk	1 cup_____
plain bagel	1 item_____
low fat cream cheese	2 tbsp_____
orange juice	1 cup_____
Lunch	
macaroni & cheese	2 cups_____
banana	1 item_____
Dinner	
baked potato	1 item_____
light sour cream	2 tbsp_____
margarine	2 tbsp_____
shredded cheddar cheese	2 oz_____
broccoli spears	1 cup_____
Harvest Burgers	2 items_____
hamburger buns	2 items_____
Snacks/Other	
low fat yogurt	1 cup_____

Subject #6-Food Checklist

Day 4

Breakfast	
whole eggs (for omelet)	2 items_____
margarine (for cooking omelet)	1 tbsp_____
shredded cheddar cheese	2 oz_____
plain bagel	1 item_____
low fat cream cheese	2 tbsp_____
orange juice	1 cup_____
Lunch	
whole wheat bread (for sandwich)	2 slices_____
reduced fat peanut butter (for sandwich)	2 tbsp_____
jelly (for sandwich)	2 tbsp_____
unsalted twist pretzels	10 items_____
Dinner	
tossed salad	1 cup_____
low calorie Italian dressing	3 tbsp_____
linguini	2 cups (cooked)_____
spaghetti sauce	1 cup_____
dinner roll	1 item_____
margarine	1 tsp_____
Snacks/Other	
apples	2 items_____
Chips Ahoy cookies	3 items_____
skim milk	1 cup_____

Appendix H: Acute Resistance Test Data Sheets

Subject:_____

Blood/Biopsy Recording Sheet

Date _/ /

Blood Draws

Sample	Time
Pre-Exercise	
Immediate Post- Exercise	
30 minute Post-Exercise	
1 Hour Post-Exercise	

Biopsy

Sample	Time
Pre-Exercise	
1 Hour Post-Exercise	

Beverage Consumption

Time Started	
Time Completed	

Exercise Recording Sheet

Baseline Testing ____/____/____

1RM Leg Press _____ **80%** _____
1RM Leg Extension _____ **80%** _____
Leg Used _____

Experimental Testing ____/____/____

Leg Press

Repetition	Weight	Time Started	Time Completed
1			
2			
3			
4			
5			

Leg Extension

Repetition	Weight	Time Started	Time Completed
1			
2			
3			
4			
5			

Appendix I: 1 RM Log Sheet

SUBJECT 1- RM LOG SHEET

Subject: _____ **Date: _____**

Beverage: _____

Height: _____

Body Weight: _____

TRIAL	BP-		SP-		Lat P-		AC-		Leg P-		LE-		LC-	
	R	W	R	W	R	W	R	W	R	W	R	W	R	W
1														
2														
3														
4														
5														
6														
1-RM														
1														
2														
3														
4														
5														
6														
1-RM														

NOTES:_____
_____
_____
_____

Appendix J: Recruitment Flyer

Nutrition and Exercise Research Study

Dept. Human Nutrition, Foods, and Exercise
338 Wallace Hall
Virginia Tech
Blacksburg VA 24061

Wanted:

Males, 18 – 25 years old
Available Spring Semester

Will involve:

- Total of 12 weeks
- Exercise training program
- Blood samples
- Muscle samples
- Urine sample

Will get:

- $200
- Strength Analysis
- Body Composition Analysis
- Personal exercise program

TO SEE IF YOU QUALIFY, contact Lauren Goldman at lgoldman@vt.edu

Lauren Goldman
lgoldman@vt.edu

References

1. Balon, T.W., A. Zorzano, J.L. Treadway, M.N. Goodman, and N.B. Ruderman. Effect of insulin on protein synthesis and degradation in skeletal muscle after exercise. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E92-E97, 1990.

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5. Biolo, G., S.P. Maggi, B.D. Williams, K.D. Tipton, and R.R. Wolfe. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E514-E520, 1995.

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