THE EFFECT OF POST EXERCISE NUTRITION ON ANABOLIC RESPONSE TO RESISTANCE EXERCISE

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ABSTRACT

Purpose: To determine the effect of four postexercise beverages, differing in macronutrient content, on metabolic response to an acute resistance exercise bout. **Methods:** Forty male subjects performed five sets of eight repetitions at 80% 1RM for leg press and leg extension, and then consumed one of four postexercise beverages (*Placebo*, PL: *a carbohydrate-electrolyte beverage*, CE; or *one of two milk-based beverages*, MILK 1: 1% chocolate milk; MILK 2: a high protein milk beverage). Indicators of muscle protein synthesis (MPS) were assessed before and 1-hr after consuming a postexercise beverage. Muscle protein degradation (MPD) was examined the day before and the day of exercise. **Results:** No significant differences were found among groups in MPS. The resistance exercise bout increased the amount of eIF4E•eIF4G by 4.5% 1-hr postexercise ($p<0.05$) without affecting the amount of eIF4E•4E-BP1. One hour after beverage consumption, serum total amino acid concentration increased for MILK 1 ($p=0.003$) and MILK 2 ($p<0.001$) but decreased for CE ($p=0.028$) and PL ($p=0.276$). Consumption of MILK 1, MILK 2, and CE significantly increased circulating levels of serum insulin ($p<0.001$). Serum growth hormone increased 3-fold as a result of the exercise bout but fell to baseline for all groups by 60 min ($p<0.001$). **Conclusion:** The resistance exercise bout was anabolic as shown by the increase in the active eIF4E•eIF4G complex and serum growth hormone. Consumption of MILK 2 led to the most optimal environment for muscle anabolism; however, none of the experimental beverages influenced the measured indicators of muscle protein translation 1-hr after ingestion. **Keywords:** MUSCLE PROTEIN

SYNTHESIS, MUSCLE PROTEIN DEGRADATION, EUKARYOTIC INITIATION FACTORS, INSULIN, GROWTH HORMONE, CARBOHYDRATE, PROTEIN

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Chapter 1:

Introduction

The ideal body for a male in today's society consists of high muscle content and low percentage of body fat. This ideal image has increased the popularity of resistance training. It is common knowledge that a program of resistance training can lead to significant increases in muscle strength and size. Individuals are willing to try just about anything if it promises to hasten the process of this muscle accretion. A survey in 1992 showed that the most frequently promoted health benefit of dietary supplements on the market was muscle growth (Philen et al. 1992). This market has continued to grow steadily. Thus, a nutritional approach designed to accelerate gains in lean body mass (LBM) without the use of expensive supplements could be extremely popular.

Statement of the Problem

It is well known that resistance exercise is a regulator of protein synthesis, increasing muscle mass (Biolo, 1993 & Luthi, 1986). Carraro et al. (1990) demonstrated that the greatest gains in skeletal muscle protein occur in the recovery period after resistance exercise. Since protein is the foundation of muscle and amino acids are the building blocks for protein, it makes sense that a supply of amino acids is necessary following resistance training to ensure optimal protein synthesis.

Many suggest that insulin also plays a role in the regulation of protein synthesis. Zorzano et al. (1985) suggested that insulin aids in muscle accretion by stimulating amino acid transport. Biolo et al. (1993) concluded that insulin has the ability to inhibit skeletal muscle protein degradation (MPD) following resistance exercise. Bennet et al. (1990) suggested that insulin, in the presence of sufficient amino acids, might stimulate protein balance by stimulating skeletal muscle protein synthesis (MPS) and inhibiting MPD. Insulin alone is not enough to increase MPS. When looking at exercised and

nonexercised rats, Fluckey et al. (1996) found that insulin only increased MPS in the exercised rats. Since insulin levels are influenced by macronutrient intake, consumption of the proper nutrients after exercise may enhance MPS and inhibit MPD. The ideal postexercise beverage would both raise the concentration of serum insulin and supply the amino acids necessary for MPS. This effect would be beneficial to all resistance trainers seeking accretion in lean body mass.

The effect of exercise on MPD has been studied extensively. However, very conflicting results are seen within the vast body of literature. MPD appears to be dependent on the type, intensity, and duration of the exercise. Supplying nutrients immediately postexercise may also alter MPD. It is necessary to study the effect of both an acute bout of high-intensity resistance exercise and the consumption of postexercise beverages differing in macronutrient composition.

Greater accretion of muscle has not been observed with a chronically higher protein intake, but the key may be the timing of intake after exercise. There may be a short time after exercise where the supply of nutrients would be optimal for promotion of muscle accretion. Research has been done on the benefits of consuming carbohydrate and amino acids postexercise; however, the effect of consuming high quality protein with carbohydrate after resistance exercise is unknown. Milk is a product that contains both carbohydrates and high quality protein, possibly making it the optimal post-exercise beverage for gaining skeletal muscle mass.

Objectives

- To determine whether serum growth hormone (GH), insulin, glucose, and amino acids (AA) 1-hr after exercise differ between groups fed carbohydrate electrolyte (CE), a low fat milk (MILK 1), a high protein nonfat milk (MILK 2), or a placebo after a resistance exercise bout.
- To determine the effect of all four beverages consumed immediately following resistance exercise on the indicators of MPD (urinary 3MH).
- To determine the effect of each beverage consumed immediately following resistance exercise on the indicators of MPS (eukaryotic initiation factors).

Hypotheses

Ho: Urinary 3-Methylhistidine will not be different among the four beverage groups on the day of resistance exercise.

Ho: Serum glucose response to beverage ingestion will not be different among the four beverage groups on the day of resistance exercise.

Ho: Serum insulin response to beverage ingestion will not be different among the four beverage groups on the day of resistance exercise.

Ho: Serum GH response to beverage ingestion will not be different among the four beverage groups on the day of resistance exercise.

Ho: Serum AA response to beverage ingestion will not be different among the four beverage groups on the day of resistance exercise.

Ho: eIF4E•eIF4G complex response to beverage ingestion will not be different among the four beverage groups on the day of resistance exercise.

Ho: eIF4E•4E-BP1 complex response to beverage ingestion will not be different among the four beverage groups on the day of resistance exercise.

Basic Assumptions

The following assumptions were made in the study:

- Subjects refrained from exercise for 3 days prior to urine collection.
- Subjects refrained from meat consumption during the days of urine collection.
- Subjects followed the instructions for urine collection and the collection was a complete 24-hr collection.
- Subjects fasted during the 12 hr prior to their acute resistance exercise test.
- Subjects honestly reported their dietary intake for the 5 days it was recorded.

Delimitations

The following delimitations were established for this study:

- Subjects were males, 18-25 years of age, who resided in surrounding area of Blacksburg, Virginia or were Virginia Tech students
- Subjects had not resistance trained for at least 3 months.
- Four beverages were used: a flavored non-caloric beverage, a carbohydrate-electrolyte sports drink consisting of 1.25 $g \cdot kg^{-1}$ of carbohydrate (CE), a milk beverage consisting of 0.92 $g \cdot kg^{-1}$ carbohydrate, 0.21 $g \cdot kg^{-1}$ protein, and 0.06 $g \cdot kg^{-1}$ fat (MILK 1), and another milk beverage consisting of 0.875 $g \cdot kg^{-1}$ carbohydrate and 0.375 $g \cdot kg^{-1}$ protein (MILK 2).
- The exercise bout designed to influence muscle protein metabolism consisted of five sets of eight repetitions of leg press and single-leg leg extension at 80% of the subjects' 1-RM.
- Subjects' dominant leg was used for both biopsies.
- All muscle samples were obtained from the vastus lateralis muscle.

Limitations

The following limitations may be present in this study:

- Results may be generalized only to individuals of similar gender, age, and training status.
- Consumption of a meat-free diet may have been necessary for additional days prior to the first urine collection
- Conclusions can be made only with regard to the exercise bout used.
- Estimation of MPD with urinary 3MH is not specific to the leg muscles used in the exercise bout.
- Using the eIF4 complex to determine MPS may not accurately reflect the change in MPS in humans.
- Since amino acids concentration was not measured immediately postexercise, the value measured 1-Hr postexercise represents the combination of the resistance exercise bout and the treatment.
- Changes in blood or muscle factors at times other than those sampled are not known.

Definitions and Symbols

- **1RM** — **(**1-Repetition Maximum) the greatest amount of weight that can be lifted with proper technique for only one repetition.
- **3MH** — **(**3-Methylhistidine) a minor amino acid found exclusively in actin and myosin, and upon its release from protein it is neither reincorporated into protein nor catabolized.
- **EAA** — (Essential Amino Acids) nine amino acids that cannot be synthesized by the human body; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine
- **BCAA** — (Branched-Chain Amino Acids) leucine, isoleucine, and valine
- **eIF4** — (Eukaryotic Initiation Factor 4) accessory proteins that aid in skeletal MPS.
- **eIF4E** — Plays a crucial role in maintaining the overall rate of MPS as well as the selection of which mRNA molecules will be translated will be translated into protein. This is done by binding to the m^7GTP cap structure present at the 5'-end of all eukaryotic mRNA.
- **eIF4A** — Thought to play a role in unwinding secondary structure at the 5'-end of mRNA to allow the 40S ribosomal subunit to bind to the 5'-untranslated region of the mRNA.
- **eIF4G** — Acts as a bridge between mRNA and the 40S ribosomal subunit.
- **eIF4E•eIF4G** — The active complex associated with MPS. Coordinates the binding of the 40S ribosomal subunit to mRNA.
- **eIF4F** — A second active complex associated with MPS. It is comprised of eIF4E, eIF4G, and eIF4A. Also coordinates the binding of the 40S ribosomal subunit to mRNA.
- **4E-BP1** — A binding protein that binds to eIF4E to inhibit MPS. It is also referred to as PHAS1.
- **eIF4E•4E-BP1** — The inactive complex associated with decreased MPS.

- **FSR** Fractional Synthesis Rate
- **Hyperaminoacidemia** The presence of excess amino acids in the blood.
- **Hyperinsulinemia** The presence of elevated insulin levels in the blood.
- **KIC** α- ketoisocaproate
- **MPD** Muscle Protein Degradation
- **MPS** Muscle Protein Synthesis

Chapter 2:

Review of Literature

Introduction

Muscle accretion can only occur if muscle protein synthesis (MPS) is greater than muscle protein degradation (MPD). Resistance training has been shown to have a dramatic effect on protein metabolism and muscle growth. It is also known that amino acids are needed for synthesizing muscle protein. Although muscle protein metabolism has been heavily studied, there are still questions about composition and timing of nutrient intake for optimal MPS and MPD. Literature related to resistance exercise, nutrient intake, and their combined effects on MPS and MPD is reviewed in this section.

Effect of resistance exercise on muscle protein synthesis and degradation

Research has demonstrated that resistance exercise increases both MPD and MPS. Chesley et al. (1992) used 12 healthy males to examine the extent and time frame for acute alterations in MPS following a single resistance exercise bout. Biopsies, from both the exercised and non-exercised arms, and blood samples were taken from group A 4 hr after exercise and 24 hr after exercise for group B. The researchers used labeled leucine infusion and examined the levels of plasma α-ketoisocaproate [^{13}C] (KIC). They found that MPS increased by 50% for group A and 109% for group B. Biolo et al. (1995b) studied the alterations in muscle protein kinetics and in transport of amino acids after a heavy, resistance exercise routine. The five subjects served as their own control by having resting samples taken 1 month prior to exercise. Blood samples were taken before and after the infusion of amino acid tracers. The exercise protocol took approximately an hour, and the infusion process was completed 3 hr after exercise. At the end of the resistance exercise, the first muscle biopsy was taken to measure phenylalanine enrichment in the muscle. The second sample was taken 3 hr later, revealing that MPS increased by approximately 108% and MPD increased by about 51%. Therefore,

resistance exercise caused an improvement in net muscle protein balance. Phillips et al. (1997) supported these findings when they found mixed muscle protein fractional synthesis rate (FSR) was elevated by 112% and mixed muscle protein fractional breakdown rate (FBR) was elevated by 31% 3-hr postexercise. One possible explanation for the vast difference in MPS results is that Chesley et al. (1992) used leucine incorporation to determine MPS, while Biolo et al. (1995b) and Phillips et al. (1997) used phenylalanine utilization. These studies show that resistance training alone affects the rates of both MPS and MPD. However, without nutrient intake to replace the energy expended, the net protein balance will remain negative.

Phillips et al. (1999) performed another similar study using phenylalanine infusions to measure the response of muscle protein FSR and FBR after an acute resistance exercise bout. But, for this study they compared trained and untrained subjects. In the untrained subjects, FSR increased by 123% and FBR increased by 37%. For the trained subjects, FSR increased by 49% and there was no significant change in FBR. The results found by Phillips et al. (1999) suggest that repeated resistance training reduces muscle protein turnover after resistance exercise. Since these researchers used the infusion technique to determine MPS, it was not measured until at least 3 hr after the completion of exercise. It would be beneficial to know the immediate effect resistance exercise has on MPS.

Researchers have used a resistance-training model for rats to observe muscle protein synthesis (Farrell et al. 1998, 1999, 2000, and Fluckey et al. 1995, 1996). By training a rat to stand up and touch an illuminated bar, researchers can have the rat perform resistance exercise similar to a squat by putting a weighted Velcro vest on the rat. The control rats perform the same movement, but without the extra weight. Both Farrell and Fluckey used this resistance-training model. Fluckey et al. (1996) measured

the rates of protein synthesis via incorporation of [^{3}H] phenylalanine into muscle protein by using an in vivo flooding dose protocol. Rates of protein synthesis were measured in both the soleus and the gastrocnemius 16 hr postexercise. Protein synthesis was 39% higher in the soleus and 92% higher in the gastrocnemius of the exercise group versus the control group. Farrell et al. (2000) also measured protein synthesis 16 hr postexercise. For this study, the researchers assessed eIF4E•eIF4G, eIF4E•4E-BP1, the phosphorylation state of eIF4E and 4E-BP1, and [^{3}H] phenylalanine incorporation in the gastrocnemius. Using [^{3}H] phenylalanine incorporation, protein synthesis was increased in the exercise group versus the control group by 25%. There were no significant changes in any of the components of the eIF4E family, but there was a trend for an increase in eIF4E•eIF4G by 37% (P=0.09).

Effect of resistance exercise on 3MH excretion

Several researchers examined the effect of resistance exercise on excretion of urinary 3MH. There have been many conflicting results, not giving a clear explanation as to the fate of 3MH following exercise.

Horswill et al. (1988) examined 3MH excretion after an acute resistance-exercise bout. Nine subjects consumed a self-selected meat free diet for 6 days. On the fifth day, the subjects performed four sets of nine exercises to fatigue at intensities of 80%, 60%, and 40% 1-RM for each successive set. Elevated muscle soreness was reported at 1 and 2 days postexercise, but 3MH did not change from the baseline value for 1 or 2 days postexercise. The authors concluded that either there was no increase in protein breakdown with resistance exercise or that urinary 3MH is not a good indicator of muscle damage from resistance exercise. Hickson et al. (1986) also found no change in urinary 3MH excretion after two resistance-training bouts designed to work both upper and lower

body muscle groups. The researchers then concluded that isolated bouts of weight lifting do not affect skeletal muscle protein catabolism. However, the findings of other studies contradict this opinion (Pivarnik et al. 1989, Viru and Seli, 1992).

Some studies have demonstrated 3MH excretion increases after exercise. Pivarnik et al. (1989) examined 3MH excretion during a 12-day resistance-training program in untrained subjects. The subjects consumed a meat-free diet 1 wk before the training program and continued it throughout the program. This routine involved four sets of five exercises at 60-70% 1RM and alternated exercises for the upper and lower body on successive days. If these researchers only examined the first 2 days of the resistance training period, their results would have supported the findings of both of the previously mentioned studies (Hickson et al. 1986, Horswill et al. 1988). However, the researchers continued to examine the effect of resistance training on 3MH finding that 3MH gradually increased over the training period, becoming significant after the third day of weight training. The authors concluded that their results supported an increase in skeletal muscle breakdown with strength training.

Viru and Seli, (1992) performed another study examining the relationship between the intensity of resistance training and 3MH excretion. During an 8-wk training period, they examined four groups; two (groups 1 & 2) that trained with high-speed movements and two (groups 3 & 4) that used a slower speed of contraction. Groups 1 and 3 used 50% of their 1RM and groups 2 and 4 used 70% of their 1RM. Six sets of four to six repetitions were performed for upper and lower body exercises. Urine samples were collected 6 hr before, 6, 12, 24, 36, and 48 hr after the training session. During the first week of training, 3MH was significantly increased at 12 and 24 hr postexercise for both 50% and 70%, with no significant differences between the two 1RM percentages. The 3MH content was determined by a method based on the reaction

with ninhydrin-orthophthaladehyde. During the first 3 wk, 3MH excretion was elevated for all groups, showing that resistance training increased 3MH excretion. During the first week, increasing the intensity yielded a 12% increase in 3MH excretion. The authors concluded that the increased excretion of 3MH shows enhanced turnover in contractile proteins, which is a necessary part of hypertrophy.

Studies have examined 3MH excretion after both aerobic and resistance exercise (Calles-Escandon et al. 1984, Dohm et al. 1982, 1985, Hickson et al. 1986, Horswill et al. 1988, Pivarnik et al. 1989, Rennie et al. 1981, Radha and Bessman 1983, & Viru and Seli 1992). Many factors, such as the type, intensity, and duration of exercise, affect the excretion of 3MH after exercise. The effect of these three variables is a reason there have been conflicting results in the studies described. Further research is needed to examine the effect of resistance exercise designed to promote hypertrophy on 3MH excretion.

Effect of food consumption on muscle protein synthesis and degradation

Food consumption is known to profoundly affect both MPS and MPD. Svanberg et al. (1996a) used eight healthy males to examine the effect of serum amino acids on MPS and MPD. A constant infusion of L- [*ring*-$^{2}H_{5}$] phenylalanine in saline was given for 8 hr along with a crystalline balanced amino acid solution. The amino acid solution was adjusted so that the nitrogen level was doubled every 2 hr. The researchers also took blood samples at the end of every 2-hr period to examine glucose, insulin, IGF-I, and plasma 3-methylhistidine (3MH). Svanberg et al. (1996a) found that the infusion of amino acids was successful in increasing plasma amino acid concentrations, without affecting plasma concentrations of glucose, insulin, IGF-I, or 3MH. Protein synthesis increased linearly with increasing doses of amino acid infusions. Protein synthesis was 70% higher with the highest dose of amino acids compared to the lowest dose. The

decrease seen in protein degradation was of the same magnitude as amino acid doses increased. However, this decrease in degradation was completely limited to globular proteins. Svanberg et al. (1996a) suggested that the improvement in protein balance seen was due to stimulation of MPS without a change in MPD of mixed skeletal muscle proteins.

Volpi et al. (1998) evaluated how peripheral infusion of amino acids in the postabsorptive state influence amino acid transport, skeletal MPS, and MPD. The researchers found a significant increase in amino acid delivery to the leg increased amino acid transport and protein synthesis. Protein degradation did not change, thus achieving a positive balance of amino acids transported across the muscle. Using both phenylalanine and lysine, Volpi et al. (1998) found that there was no change in protein synthesis efficiency, indicating that amino acids alone can stimulate MPS by increasing inward amino acid transport.

Volpi et al. (1999) performed a similar study, but used an oral dose of amino acids and also measured the effect of the amino acids on insulin concentration. After the oral dose of amino acids, arterial amino acid concentration increased by 63%, plasma insulin concentration increased by 73% and protein synthesis increased by 80% compared to basal values. The conclusion of these authors was that muscle protein anabolism was stimulated by the increase in availability of amino acids. All three of these studies found the provision of amino acids led to an increase in MPS with one study suggesting that this was facilitated by the elevated blood insulin concentration and another study finding no change in insulin concentration.

Yoshizawa et al. (1998) examined the effect of dietary protein on translation initiation in rat skeletal muscle. The researchers compared rats that had been food deprived for 18 hr to those fed isocaloric diets containing either 20% protein or 0%

protein. A 38% increase in MPS was seen in the 20% protein group compared to the fasting rats, with no change occurring in the 0% protein group. The increase seen in MPS was associated with increased phosphorylation of 4E-BP1, reduced binding of 4E-BP1 with eIF4E, and increased formation of eIF4E•eIF4G. The 0% protein diet had no effect on these factors. Both diets increased plasma insulin 2.5-fold in comparison to the rats in the fasting state, leading the authors to the conclusion that an increase in insulin is not enough for stimulation of MPS. A combination of dietary protein and an increase in insulin concentration may be required to promote translation initiation.

Anthony et al. (2000a, 2000b) examined MPS in postabsorptive rats. For both studies, the researchers used rats that had been deprived of food for 18 hr. Anthony et al. (2000a) investigated the MPS response 1-hr after administering an oral dose of leucine alone, carbohydrate alone, and a combination of leucine and carbohydrate and compared these groups to a group only given saline. Administration of carbohydrate alone did not affect MPS, but MPS increased by 36% and 38% when leucine was administered alone or in combination with carbohydrate. Compared to the food-deprived group, the amount of 4E-BP1 associated with eIF4E was unchanged when the rats were fed carbohydrate alone, but was reduced by 70% with the administration of leucine and 60% with the combination of leucine and carbohydrate. The amount of eIF4G associated with eIF4E was unchanged when the rats were fed carbohydrate alone, but was increased by 208% with the administration of leucine and increased by 115% with the combination of leucine and carbohydrate when compared to the food-deprived group. To further investigate MPS, Anthony et al. (2000a) examined insulin concentrations for the groups. A 2.6- and 3.7-fold increase in insulin concentration was found with the oral administration of carbohydrate or combination of carbohydrate and leucine when compared to the saline group. The administration of leucine alone had no affect on

insulin concentration, indicating that leucine stimulates MPS by enhancing eIF4E•eIF4G formation independently of increases in serum insulin.

The purpose of the second study performed by Anthony et al. (2000b) was to investigate whether leucine has a greater stimulatory effect on MPS in food-deprived rats than other BCAA. MPS was examined 1-hr after fasting rats were administered saline or 270 mg of valine, isoleucine, or leucine. Serum insulin concentrations were not affected by administration of any of the BCAA. Of the three BCAA, leucine was the only one to stimulate MPS, showing a 65% increase in MPS compared to the control rats. The amount of the inactive eIF4E•4E-BP1 complex in the rats fed leucine was 17% of the amount in the control rats. Also, when comparing rats fed leucine to the control rats, the active eIF4E•eIF4G complex was 4-fold greater and 4E-BP1 phosphorylation was 5-fold greater in the leucine fed rats. Anthony et al. (2000b) concluded that leucine was unique in its ability to stimulate MPS and it was the most effective among BCAA in augmenting translation initiation.

Vary et al. (1999) found that supraphysiological amino acid concentrations (10x physiological concentrations) lead to a 2-fold increase in protein synthesis and the formation of the active eIF4E•eIF4G complex increased by 8-fold, without a change in the inactive complex. Vary et al. (1999) then compared rats given the supraphysiological amino acid perfusate to rats given a supraphysiological amino acid perfusate minus leucine. When leucine was removed, MPS decreased by 40%; eIF4E•4E-BP1 complex increased 3.6-fold; and the eIF4E•eIF4G complex decreased 80%. These results imply that leucine is an integral factor in the stimulation of protein synthesis. Vary et al. (1999) concluded that amino acids not only increase MPS by acting as precursors, but it appears that they play a regulatory role as well, promoting translation initiation by augmenting the binding of eIF4E to eIF4G.

Carbohydrate has also been proposed to improve the net protein balance. In a study by Krempf et al. (1993), subjects were either given a diet infusion providing 83 $mg \bullet kg^{-1} \bullet hr^{-1}$ amino acid or a combination of this amino acid solution and 0.167 $g \bullet kg^{-1} \bullet hr^{-1}$ Polycose for 6 hr via a feeding tube placed in the duodenum. During the last 4 hr of the diet infusion, labeled tracers were infused via I.V. and the feeding tube. Blood samples were taken before the infusion of labeled tracers and seven times during the last 90 min. The researchers found the addition of dietary carbohydrate with amino acids decreased the rate of leucine release from tissue and organ protein breakdown by approximately 29.1 $\mu mol \bullet kg^{-1} \bullet hr^{-1}$ compared to amino acids alone. Leucine oxidation also decreased by approximately 6.8 $\mu mol \bullet kg^{-1} \bullet hr^{-1}$. Carbohydrate has a sparing effect on dietary leucine utilization and body leucine metabolism. Proposed to be mediated by insulin, dietary carbohydrate acted to decrease MPD, thus lowering the plasma leucine concentration. This reduction lowers leucine oxidation, sparing it for anabolic purposes. Also splanchnic output of dietary leucine increased, favoring maintenance of protein synthesis. Both exogenous amino acids and carbohydrates have been shown to promote anabolism, amino acids by increased MPS and carbohydrates by decreased MPD. A combination of both macronutrients may be the most beneficial for improving overall muscle protein balance.

Effect of diet manipulation with resistance exercise on muscle protein synthesis

Since both diet manipulation and resistance exercise individually affect MPS, the combination of diet manipulation and resistance exercise may have an even greater effect than the two variables alone. As stated earlier, Biolo et al. (1995b) found that the net increase in protein synthesis was dependent on amino acid delivery to the muscle increasing by 80-110%. Thus, the availability of amino acids can be a limiting factor for

improvement of net muscle protein balance. An ideal combination may be provision of exogenous amino acids after a resistance exercise stimulus. Biolo et al. (1997) used an infusion of a balanced amino acid mixture for 3 hr at rest and after a leg-resistance exercise bout. Subjects were first tested at rest and then 1-4 weeks later after exercise. The leg-resistance exercise bout consisted of five sets of 10 repetitions of incline leg press and four sets of eight repetitions of Nautilus duo-squat, leg curls, and leg extensions. The amino acid infusion occurred during hours 4-7, successfully inducing hyperaminoacidemia. For the exercise group, this was initiated 1-hr postexercise. The researchers found that during hyperaminoacidemia, protein synthesis was approximately 150% higher after exercise than rest and the rate of transport of amino acids into the muscle cell was 40-100% greater after exercise than at rest. This suggests that anabolism will be greater if resistance exercise is paired with hyperaminoacidemia.

The technique of infusion is not a practical way for individuals to receive the necessary amino acids after exercise. Therefore, the same laboratory examined an oral dose of amino acids (Tipton et al. 1999). Their subjects were given constant infusion of L- [*ring*-$^{2}H_{5}$] phenylalanine for 390 min in order to measure protein metabolism. The exercise bout, as described in the previous study, was performed an hour after the start of the infusion. After the intense leg-resistance exercise bout, their subjects ingested 1 L of the test solution at a rate of 100 mL every 18-20 min. The 1-L solutions contained either 40 g of mixed amino acids, 40 g of essential amino acids, or a placebo of double-distilled water with an artificial sweetener. Blood samples were taken at the beginning, before the consumption of the beverage, and three times during the last 45 min. A muscle biopsy was also performed at 270 min postexercise. Their results showed that an oral dose of amino acids is just as effective as infusion at inducing hyperaminoacidemia. The rate of phenylalanine utilization for the mixed amino acid group and essential amino acid group

was approximately 70% and 50% higher than the placebo, with no significant differences found between groups for the rate of protein breakdown. However, the net muscle protein balance was significantly higher for the mixed amino acid group and essential amino acid group as compared to the placebo, with the essential amino acid group having the most positive balance. Therefore, they concluded that only the essential amino acids are needed to promote anabolism.

The results of this experiment prompted another study by the same laboratory to determine the muscle protein response to ingestion of a bolus containing both essential amino acids and carbohydrate after resistance exercise (Rasmussen et al. 2000). Six subjects, examined twice so they could serve as their own control, performed an exercise protocol of 10 sets of eight repetitions for leg press and eight sets of eight repetitions for leg extension at 80% 1RM. 1-hr after the exercise bout, the subjects received either an essential amino acid-carbohydrate drink or a placebo. At 3 hr postexercise, they received the opposite drink. The experimental drink contained 6 g of essential amino acids and 35 g of sucrose dissolved in 500 mL distilled water, along with a small amount of artificial sweetener to enhance taste. The placebo was an artificially flavored drink containing a small amount of aspartame. A constant infusion of $[^{15}N]_2$ urea was started 90 min before exercise and L- [*ring*-$^2H_5$] phenylalanine 60 min prior to exercise. Both were continued throughout the experiment. Ten blood samples were taken during the 4-hr period after exercise and muscle biopsies were taken prior to the first drink and an hour after each drink. The drink containing essential amino acids and carbohydrates was effective in increasing arterial phenylalanine and insulin concentrations, the net balance of phenylalanine across the leg, and MPS when ingested at either time point. The authors concluded that the beverage with essential amino acids and carbohydrates promoted muscle anabolism whether fed at 1-hr or 3-hr postexercise. They also suggested that

future studies needed to be done to examine if the ingestion of amino acids immediately postexercise would be as successful at promoting anabolism.

Roy et al. (1997) examined the effect of glucose supplementation following resistance exercise on FSR. Immediately after and 1-hr after performing eight sets of 10 repetitions of knee extensions at 85% of 1RM, subjects received either $1g \cdot kg^{-1}$ carbohydrate supplement or a placebo. Insulin was significantly greater in the experimental group from 0.5 to 2 hr postexercise. Neither beverage had a significant effect on FSR, suggesting that supplementation of carbohydrate alone immediately after resistance exercise is not enough to promote MPS.

More recently Roy et al. (2000) examined the effect of postexercise beverages with differing macronutrient composition on whole body protein synthesis following resistance exercise. For this study, subjects performed three sets of 10 repetitions of exercises targeting all major muscle groups at ~80% of 1RM. The carbohydrate supplement and placebo were similar to their previous study (Roy et al. 1997). The third beverage was isocaloric to the carbohydrate beverage, but was ~66% carbohydrate, ~23% protein, and ~12% fat. Supplements were given immediately postexercise and 1-hr postexercise. Whole body protein synthesis, as estimated by nonoxidative leucine disposal (NOLD), significantly increased by 41% in the carbohydrate/protein/fat group and by 33% in the carbohydrate only group. This contradicts their previous study which found carbohydrate alone was not enough to stimulate MPS. The difference seen between the two studies is possibly related to the examination of FSR vs. NOLD.

Tipton et al. (1999) stated that their study was "the first report of improving muscle protein anabolism in humans with an oral amino acid supplement after exercise." Then it was shown that the ingestion of amino acids with carbohydrates postexercise promotes anabolism. The improvement from amino acids ingested after exercise needs

further research. Studies have not been done with nutrient intake immediately postexercise. In addition, the source of nutrients needs to be examined. Instead of using amino acid supplements, which could become expensive, whole protein such as from milk needs to be tested.

Effect of diet manipulation with resistance exercise on muscle protein degradation

As with MPS, both diet manipulation and resistance exercise individually affect MPD. Perhaps the combination of diet manipulation and resistance exercise can create the optimal environment for muscle protein anabolism. Biolo et al. (1995b) found that resistance exercise increased MPD by 51%. When an infusion of amino acids was administered 1-hr after exercise, Biolo et al. (1997) found that MPD was not significantly different after exercise from the basal values, indicating that the supply of amino acids prevented the increase in MPD that normally ensues following resistance exercise. The proposed mechanism for this reduction is maintaining the cellular uptake and intracellular concentration of amino acids above basal levels, theoretically decreasing the stimulus for MPD. Rasmussen et al. (2000) supported this conclusion when the researchers found the oral consumption of amino acids after resistance exercise increased MPS without increasing MPD over the resting rates. One potential flaw in the later two mentioned studies (Biolo et al. 1997, Rasmussen et al. 2000) is that based on the results of Biolo et al. (1995b), they assumed that resistance exercise increased MPD without using a exercise-only control to test this assumption.

Several researchers measured 3MH as an indicator of MPD. Studies using this method found a reduction of 3MH excreted due to dietary intervention after an exercise bout. Roy et al. (1997) examined the effect the timing of glucose supplementation given after a bout of resistance exercise on synthesis and MPD. The subjects consumed an

isoenergetic, isonitrogenous, flesh-free diet for 3 days prior to the trial. Each subject performed four sets of eight to 10 repetitions of unilateral leg press and leg extension at 85% of their 1RM. After samples were taken, the glucose supplement (1 g · kg $^{-1}$) or placebo (NutraSweet) drink was consumed. All of the subjects were required to collect all urine excreted from approximately 12 hr before exercise to 12 hr postexercise. The urine was analyzed for creatinine, 3MH, and urea nitrogen. The researchers found a reduction in urinary 3MH and urea nitrogen excretion when carbohydrates were given after an exercise bout versus a placebo.

More recently, Roy et al. (2000) performed a study examining what effect the macronutrient composition of beverages had on muscle anabolism and catabolism following resistance exercise in trained subjects. Three beverages were tested, a placebo consisting of sucralose, a carbohydrate only, and a beverage with 66% carbohydrate, 23% protein, and 12% fat. The two test beverages were isoenergetic (4 kcal · kg $^{-1}$). The subjects performed a bout of heavy resistance training, and then consumed a beverage both immediately postexercise and 1-hr postexercise. Using a catheter, blood samples were taken immediately after exercise and every 20 min for 160 min, then again at 240 min, 255 min, and 270 min postexercise. Expired gas collections were also performed at 240 min, 255 min, and 270 min postexercise. Urinary 3MH was examined in 24-hr urine samples. 3MH excretion was not significantly different between any of the groups, conflicting with the previous study where Roy et al. (1997) found that consumption of carbohydrates immediately after resistance exercise had a significantly lower excretion of urinary 3MH than the placebo group.

Exogenous amino acids after exercise have been shown to reduce the excretion of urinary 3MH compared to resistance exercise without food consumption. Carbohydrate consumption has shown conflicting results within the same laboratory, demonstrating a

reduction in 3MH excretion with the consumption of carbohydrate and the other study showed that carbohydrate consumption had no effect on urinary 3MH excretion. The one study looking at the effect of a combination of protein and carbohydrate postexercise did not show a reduction in MPD as indicated by urinary 3MH compared to placebo consumption. Beverages containing a combination of protein and carbohydrate still need further examination.

Hormonal influences on protein metabolism

Insulin as an Anabolic Hormone

It is well established that circulating levels of certain hormones exert an anabolic effect on protein metabolism in humans. This anabolic effect likely includes modulation of both MPS and MPD. Insulin has been suggested to be a mediator in both MPS and MPD.

Bennet et al. (1990) studied eight subjects using an infusion of amino acids alone and an infusion of glucose, insulin, and amino acids, demonstrating that arterial plasma amino acid concentrations were 38% higher when insulin was infused with amino acids than when amino acids were infused alone. Also, leg net arteriovenous balance of amino acids was 590% higher when insulin was infused with amino acids than when amino acids were infused alone. The combined infusion also showed a 4-fold increase in leg leucine oxidation, an increase in whole body NOLD, a decrease in leg leucine plus KIC release, and a slight decrease in release of phenylalanine. These findings lead to the conclusion that insulin, in the presence of sufficient amino acids, might stimulate MPS and inhibit MPD.

Svanberg et al. (1996a) found that increasing concentrations of amino acids with stepwise increased amino acid infusions did not affect plasma insulin concentrations and

the provision of amino acids without insulin did not affect MPD. In contrast, Volpi et al. (1998) found a significant increase of 4 $\mu U \cdot mL^{-1}$ in insulin concentration with the infusion of amino acids. This increase in insulin was suggested to increase amino acid transport and MPS. Muscle protein anabolism appears to be promoted when there is both an increase in availability of amino acids and elevated blood insulin concentration (Svanberg et al. 1996a, Volpi et al. 1998).

Fluckey et al. (1996) performed a study to examine the effect insulin had on skeletal MPS after four sessions of resistance exercise. An in vivo flooding dose protocol was used for incorporation of [^{3}H] phenylalanine to assess rates of MPS in 12 rats. Six were rested and the other six performed acute resistance exercise. Simultaneous perfusions were performed on each leg of the rat, but only one leg received insulin. A significant increase in MPS was found in the leg given insulin of the exercised rats vs. the leg not given insulin of the exercised rats and either leg of the nonexercised rats. Insulin did not affect MPS in the nonexercised rats. The authors concluded that insulin is necessary after resistance exercise for elevated protein synthesis rates.

Biolo et al. (1999) examined the effects of insulin and exercise on the rates of MPS and MPD and amino acid transport in untrained normal volunteers. The volunteers were all studied in the postabsorptive state at rest and about 3 hr after a heavy leg resistance exercise routine. Using infusion techniques, protein synthesis and degradation were determined as rates of intramuscular phenylalanine utilization and appearance, and muscle FSR was determined. Insulin infusion increased protein synthesis at rest, but had no effect after exercise, contradicting the results found by Fluckey et al. (1996). Biolo et al. (1999) suggested that this difference might have been due to MPS being limited by amino acid availability. In addition, insulin infusion had no effect on protein degradation at rest, but significantly decreased the rate of protein degradation after exercise,

demonstrating that resistance exercise increases insulin's ability to suppress skeletal MPD.

One proposed way insulin stimulates MPS is through an enhanced initiation of translation of mRNA. This might be explained by enhanced formation of a eukaryotic initiation factor 4F (eIF-4F). eIF-4F is comprised of three proteins termed eIF4E, eIF-4A, and eIF4G. Each protein in the eIF-4F complex plays an individual role in coordinating the binding of the 40S ribosomal subunit to mRNA. eIF4E plays a critical role in the overall rate of protein synthesis. eIF4G has been suggested to act as a "bridge" between mRNA and the 40S ribosomal subunit because it contains binding sites for eIF4E and eIF4A as well as for eIF-3 associated with the ribosome (Kimball et al. 1997).

One way insulin may enhance translation initiation is by regulating the binding of eIF4E to 4E-BP1. The binding of eIF4E to 4E-BP1 creates an inactive complex that inhibits protein synthesis. In skeletal muscle, insulin causes an increased phosphorylation of 4E-BP1, causing eIF4E to be released from the inactive complex. It has been assumed that this release of eIF4E will lead to the formation of the active eIF-4F complex (Kimball et al. 1997).

Kimball et al. (1997) studied the mechanism through which insulin stimulates translation initiation in the gastrocnemius muscle of rats after a 90-minute perfusion of bovine erythrocytes, bovine serum albumin, glucose, phenylalanine, all other amino acids at normal rat plasma concentrations, and insulin. After the perfusion, the muscle was extracted and examined for eIF-4F and eIF4E•4E-BP1 complexes. The researchers also examined the phosphorylation states of eIF4E and 4E-BP1. Rates of protein synthesis were analyzed by measuring phenylalanine incorporation into the muscle. Compared with the control muscle, insulin stimulated protein synthesis 1.6-fold in perfused

gastrocnemius muscle. With the treatment of insulin, the researchers observed a 12-fold increase in the amount of eIF4G present in the eIF4E immunoprecipitate, implying that one way insulin stimulates MPS is by promoting the formation of the eIF-4F complex. A mechanism through which insulin might increase the binding of eIF4G to eIF4E is by causing the dissociation of the eIF4E•4E-BP1 complex. Once eIF4E is released from 4E-BP1, it would then be free to bind to eIF4G. Insulin caused a decrease in the total amount of 4E-BP1 that coprecipitated with eIF4E to ~40% of the control value. There is a correlation between phosphorylation of 4E-BP1 and the dissociation of the eIF4E•4E-BP1 complex. Insulin caused a 3.3-fold increase in the proportion of 4E-BP1 present in the most highly phosphorylated form, suggesting that the phosphorylation of 4E-BP1 frees eIF4E from the inactive complex and allows it to bind to eIF4G.

The role of insulin in muscle protein metabolism is still somewhat controversial. Some believe it is an important mediator of skeletal muscle anabolism, playing a role in both MPS and MPD (Fluckey et al. 1996, Kimball et al. 1997). Biolo et al. (1999) found that an insulin infusion increased MPS at rest, but not after exercise. However, Fluckey et al. (1996) and Kimball et al. (1997) found it to be essential after exercise for muscle protein anabolism. The consumption of carbohydrate or a combination of carbohydrate and protein after resistance exercise has been shown to significantly increase plasma insulin levels (Zawadzki et al. 1992, Chandler et al. 1994, Roy et al. 1998, 2000, Rasmussen et al. 2000). Rasmussen et al. (2000) also examined MPS, finding a significant increase with the beverages that raised insulin levels. If the suggested roles of insulin are valid, the most suitable environment for skeletal muscle accretion may be sufficient amino acids in the presence of elevated serum insulin.

Growth Hormone

Another hormone that is considered essential for skeletal muscle growth is growth hormone (GH). GH is a peptide hormone secreted by the anterior pituitary, which acts through somatomedin activity to regulate growth and metabolism in humans (Kraemer 1988). Human GH has shown a role in promoting MPS, assisting the transport of amino acids across the cell membrane, increasing RNA synthesis, promoting lipolysis, and promoting tissue growth via nitrogen retention (Kraemer et al. 1992, Clarkson and Thompson 1997). The GH response to resistance exercise may facilitate the creation of an optimal environment for MPS and muscle anabolism.

William Kraemer has performed multiple studies involving exercise and the endocrine system, many of which examined the effect of exercise on GH. In one of these studies, Kraemer et al. (1998b) examined the hormonal responses to consecutive days of resistance exercise. The study was a double-blind crossover design where the subjects consumed either a high-calorie liquid supplement or a placebo three times per day for 1 wk. The supplement was composed of 67% carbohydrate and 33% protein. The placebo was designed to look and taste like the supplement, but with minimal carbohydrate, protein, and calories. Exercise was performed on the last 3 days of the 1-wk period. The resistance exercise protocol consisted of four sets of 10 repetitions for squat, bent over row, bench press, and military press, with 2 min between sets. Half of the beverage was consumed 2-hr before exercise and immediately after the postexercise blood draw. Blood samples were taken preexercise, 0, 15, 30, 45, and 60 min postexercise. Serum GH was measured using a double-antibody ^{125}I liquid-phase RIA. For both the supplement and placebo group, concentrations of serum GH increased significantly immediately postexercise and returned to basal by 60 min postexercise. On day 1, serum GH concentrations were significantly higher at all postexercise times for subjects who

ingested the supplement compared to those who ingested the placebo. This difference in GH concentrations ranged from 36% to 71%. This result was not seen on days two and three. The authors concluded that many factors, including amino acid intake and the lower preexercise glucose concentration during supplementation, could have been responsible for the difference between the two groups on day 1. As in this study, other research performed by Kraemer has shown that moderate- and high-intensity resistance exercise increases serum GH concentrations (1988, 1992, 1991, 1999, 1990, 1998a).

Chandler et al. (1994) performed a study to determine whether supplementation with carbohydrate, protein, or carbohydrate-protein after resistance exercise could induce a hormonal environment favorable to MPS. Subjects performed two sets of eight exercises. For each set, subjects performed 8-10 repetitions at ~75% of their 1RM and were allowed 90 seconds of rest between sets. Blood samples were taken immediately before exercise and 0.5, 1, 2, 3, 4, 5, 6, and 8 hr postexercise. Subjects consumed one of four test beverages immediately and 2-hr postexercise. The three experimental beverages were isocaloric, providing 5.65 kcal · kg $^{-1}$ body weight. At 6 hr postexercise, all subjects received a standardized meal containing approximately 65% carbohydrate, 25% fat, and 10% protein. During the 8-hr postexercise recovery period, all subjects rested quietly but were not permitted to sleep. GH increased significantly 613% after resistance exercise, but was not affected by the experimental beverages. By 2 hr postexercise, GH had returned to baseline concentration.

Suminski et al. (1997) investigated the acute effect of ingesting a mixture of arginine and lysine on basal GH secretion. The subjects performed three sets of seven exercises at 70% 1RM until exhaustion. The exercise was performed immediately after the subjects ingested either 3000 mg vitamin C or 1500 mg arginine with 1500 mg lysine. The same two variables were used with subjects who did not exercise. Blood samples

were taken immediately prior to supplementation (time 0) and 30, 60, 90, 120, and 180 min after time 0. The resistance-exercise protocol last approximately 50 min. Exercise resulted in a significant increase in GH at 30, 60, and 90 min. Sixty minutes after supplementation, subjects who ingested amino acids without exercise had GH concentration that was 2.4-fold higher than the subjects who ingested the placebo without exercise. However, amino acid supplementation did not augment the exercise-induced GH increase when compared to the placebo.

Bucci et al. (1990) examined the effect of three amounts of oral ornithine, given in single doses, on growth hormone release in bodybuilders. The three doses of ornithine, administered in capsules, were 40, 100, and 170 mg · kg $^{-1}$ body weight. These doses were given in the fasting condition and blood samples were taken immediately before, 45 min, and 90 min after supplement ingestion. Mean serum GH levels tended to rise with ornithine ingestion, but the only significant increase (318%) was seen 90 min after subjects ingested 170 mg · kg $^{-1}$.

Lambert et al. (1993) examined the effect of commercially available amino acid supplements on serum GH concentrations in the fasting state. The three supplements were a 2.4 g mixture of arginine and lysine; a mixture of 1.1 g ornithine, 750 mg tyrosine, 750 mg pyridoxine HCl, and 125 mg ascorbic acid; and a mixture of 438 mg arginine, 412 mg lysine, 362 mg leucine, 312 mg valine, 238 mg phenylalanine, 200 mg isoleucine, and 580 mg carbohydrate. Blood samples were collected prior to supplement ingestion and then every 30 min for 3 hr. Serum GH was not affected by any of the supplements. This seeming contradiction to the results of Suminski et al. (1997) and Bucci et al. (1990) is probably due to Lambert et al. (1993) using lower doses of amino acids than the previously mentioned studies.

Studies have shown that GH promotes anabolism of skeletal muscle. Serum concentrations of these hormones can be affected by manipulation of diet and resistance exercise. Further research is still needed to determine what nutritional supplementation immediately following resistance exercise would yield optimal concentrations of this hormone.

Assessment of muscle protein synthesis

There are a couple of techniques used to measure MPS. The most common method involves both infusion of tracer amino acids and muscle biopsies. A second approach requires muscle biopsies to measure concentrations of factors that control translation in the muscle. Both of these procedures are complex and invasive. The question is which technique is the best to use following resistance exercise.

The infusion technique has been used in many research studies to measure MPS (Biolo 1992, 1995a, 1995b, 1995c, 1997, 1999, Kimball 1997, Rasmussen 2000, Roy 2000, Svanberg 1996a, 1996b, Tipton 1999). This method involves inserting a catheter into a peripheral vein for the infusion of stable isotopic tracers of amino acids. The most commonly infused amino acid is phenylalanine. Some of the tracers used are L-[ring $^{2}H_{5}$]phenylalanine (Biolo 1992, 1995a, 1995b, 1995c, 1997, 1999, Rasmussen 2000, Svanberg 1996a, Tipton 1999), L-[U- ^{14}C]phenylalanine (Svanberg 1996b), L-[ring $^{13}C_{6}$]phenylalanine (Biolo 1992, 1995a, 1995b, 1995c, 1997, 1999), and L-[1- ^{13}C]leucine (Biolo 1992, 1995a, 1995b, 1995c, 1997, 1999, Chelsey 1992). The tracers are infused evenly over an extended period ranging from 3-6 hr, dependent on the other protocol of the study. Muscle biopsies are then necessary for the measurement of intracellular amino acid enrichment using high-performance liquid chromatography (HPLC). This technique is very accurate and has been validated by several studies (Biolo 1995a, 1995b, & 1995c,

Chelsey 1992, Rasmussen 2000, Svanberg 1996a, 1996b, and Tipton 1999). However, the two most notable drawbacks to this technique are the expense and the amount of time subjects have catheters inserted for the infusion process.

In recent research, molecular biology has become an important tool in assessing the effects of diet and exercise on MPS. Transcription of mRNA for muscle proteins has been shown to be up-regulated following resistance exercise (Pette and Dusterhoft 1992). Booth et al. (1997) suggested this up-regulation could occur by augmenting the transcription of genes, but that translational and post-translational regulation is more likely to be the most crucial process following an acute bout of resistance exercise. Welle et al. (1999) ascertained that exercise stimulated myofibrillar synthesis without a change in RNA or mRNA concentrations, suggesting that this stimulation is controlled by more efficient translation of the existing mRNA.

This efficiency of translation is regulated by eukaryotic initiation factors (eIF), which act at the level of peptide chain initiation. Several investigators have quantitatively examined these eIF complexes as a measure of MPS in rats (Anthony et al. 1999, 2000a, 2001, Farrell et al. 2000, Gautsch et al. 1998, Kimball & Jefferson 2002, Vary et al. 1999, and Yoshizawa et al. 1998). The binding of the mRNA to the 43S preinitiation complex has been described as a rate-controlling step in translation initiation (Gautsch 1998). The eIF4F complex, consisting of eIF4A, eIF4E, and eIF4G, collectively serves to recognize, unfold, and guide the mRNA to the 43S preinitiation complex. The function of the eIF4F complex appears to be modulated by the ability of eIF4E to bind to eIF4G. This can be controlled by both the phosphorylation state and the availability of eIF4E. 4E-BP1 competes with eIF4G for binding eIF4E, thus affecting the availability of eIF4E for eIF4F complex formation. If eIF4E binds with 4E-BP1, it is

converted into an inactive complex. Phosphorylation of eIF4E, eIF4G, or 4E-BP1 changes the relative binding affinity, thus regulating rates of translation (Gautsch 1998).

Protein has been shown to be a required component in the diet for the stimulation of translational initiation to occur in muscle. After feeding a diet consisting of 20% protein, Yoshizawa et al. (1998) found more of 4E-BP1 to be in the phosphorylated or inactive form. This phosphorylated form of 4E-BP1 does not bind to eIF4E. By decreasing the concentration of the active 4E-BP1, there was approximately a 40% increase in the formation of the active complex, eIF4E•eIF4G. Another study examined the change in the eIF4E complexes in rats fed a carbohydrate or "complete" meal after exercise (Gautsch et al. 1998). The "complete" meal consisted of 54.5% carbohydrate, 14% protein, and 31.5% fat. Each rat underwent an 8-day acclimation period for the treadmill, with either speed or duration gradually increasing. On the ninth day, the experimental run was performed for 2 hr at 26 m/min. This intensity was approximately 75% VO_{2MAX} for the rat. The changes in eIF in the muscle were examined along with using a labeled leucine infusion to measure MPS. Protein synthesis as measured by both methods, increased only in the group with protein in their meal. This increase in synthesis was associated with a lower concentration of eIF4E bound to 4E-BP1 and a higher concentration bound to eIF4G. The fact that the change in eukaryotic initiation factors reflected the change in MPS shown by label infusion suggests that this molecular biology technique is a valid measure for MPS. Several other studies examining MPS demonstrated the validity of measuring eIF to determine changes in MPS (Anthony et al. 1999, 2000a, 2001, Farrell et al. 2000, Kimball & Jefferson 2002, Vary et al. 1999, and Yoshizawa et al. 1998).

Using infusion to determine MPS is a very accurate technique. However, it is an expensive method that involves a greater risk for the subjects. Not only are muscle

samples taken, but also a solution is being infused into their bloodstream, presenting a higher risk of infection. Another negative aspect of using infusion is the amount of time required. The subject has to remain in the laboratory for a minimum of 3 hr, sometimes over 6 hr.

Measuring eIF in muscle from human subjects is more difficult than using rats. The majority of studies using eIF use rats because the whole muscle is available for examination. Measuring eIF in human samples is more complex because it requires the miniaturation of the process used with rat muscle, since only small muscle samples can be taken for testing. It should also be noted that since the previous studies involved rats, it is possible that the binding of the mRNA to the 43S preinitiation complex is not a rate-controlling step in translation initiation for humans. It is also possible that the activity of another complex, such as eIF2B instead of eIF4E•eIF4G, is more indicative of changes in MPS in humans. Examining eIF to measure MPS eliminates some risk for the subjects since solutions are not infused and measuring eIF is more comfortable for the subject than infusion since the subject is not connected to a machine for many hours. If examination of eIF in human muscle is demonstrated to be a valid measure of MPS, it should be used over tracer infusion.

Assessment of muscle protein degradation

Two procedures have been predominantly used to assess MPD; isotopically labeled amino acid infusion paired with muscle biopsies and measuring total excretion of 3MH in the urine.

As previously discussed for measuring MPS, using infusion is a laborious, time-consuming technique. It is also much more expensive and presents more risk to the subjects than measuring urinary 3MH. The use of urinary 3MH as an indicator of muscle

protein breakdown is attractive due to its noninvasive nature. However, there has been some controversy as to whether it is a valid indicator of muscle protein breakdown. Individuals opposed to using 3MH cite its excretion from sources other than skeletal muscle (Rennie and Millward, 1983). High protein turnover in the gastrointestinal tract and skin may inflate 3MH values. Another problem with 3MH is dietary control. Meat contains inconsistent but substantial levels of 3MH. Since it cannot be incorporated for MPS, it is excreted in the urine. Unless this is controlled for, urinary 3MH measurements will be inconsistent. To avoid this inconsistency, subjects need to consume a meat-free diet prior to and throughout testing. Many believe that a meat-free washout period is necessary, but a timeframe for this period has not been determined. Previous researchers have directed their subjects to consume a meat-free diet for as little as 2 days prior to exercise to the entire week before the exercise period (Calles-Escandon et al. 1984, Hickson et al. 1986, Horswill et al. 1988, Pivarnik et al. 1989, Radha and Bessman 1982, and Viru and Seli 1992). Pivarnik et al. (1989) examined 3MH excretion every day during both the washout and experimental periods. There were no significant differences in urinary 3MH for any day during the washout period, but there was a slight decrease from day 1 to day 2 followed by very consistent concentrations. Therefore, it would be best to use a meat-free diet for a minimum of 1 day prior to measuring 3MH.

Ballard and Tomas (1983) argue in favor of continuing the use of 3MH to measure MPD, estimating that 91% of 3MH excretion is derived from skeletal muscle. By using a 70 kg reference man with 30 kg of skeletal muscle and taking into account known quantities of 3MH measured in μmol/g protein for tissues such as the heart, liver, and lungs, Ballard and Tomas (1983) calculated that the gastrointestinal tract contributed 3%, the skin 3%, and other internal organs less than 1% each. Long et al. (1988) also examined the role that the gastrointestinal tract plays in urinary 3MH excretion by

evaluating 3MH excretion among patients that had a massive small-bowel resection, in which approximately 72% of the gastrointestinal tract was removed. If the gastrointestinal tract contributes a significant amount of 3MH, then the patients with shortened gastrointestinal tracts would have decreased 3MH excretion. However, the researchers found 3MH excretion after the massive small-bowel resection was not significantly different from the values taken before surgery. A conclusion can be drawn that urinary 3MH excretion is a reasonable representation of muscle protein breakdown and can be useful to determine the effect of different interventions on muscle protein breakdown.

As discussed in previous sections, acute exercise appears to have an effect on the excretion of urinary 3MH (Pivarnik et al. 1989, Viru and Seli 1992). The benefits of using 3MH excretion include it being less expensive than the infusion and a non-invasive procedure. Thus, assuming that intake of meat is tightly regulated, the practice of measuring total excretion of 3MH is beneficial for researchers to use as an indicator of MPD.

Chapter 3:

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Effect of Postexercise Nutrition on Anabolic Response to Resistance Exercise

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Abstract

Purpose: To determine the effect of four post-exercise beverages, differing in macronutrient content, on metabolic response to an acute resistance exercise bout. **Methods:** Indicators of muscle protein synthesis (MPS) and muscle protein degradation (MPD) were assessed in 40 males before and 1-hr after the consumption of a postexercise beverage. Subjects performed five sets of eight repetitions at 80% of 1RM for leg press and leg extension, and then consumed one of four postexercise beverages. (*Placebo*, PL: a calorie-free beverage; *a carbohydrate-electrolyte beverage*, CE: 5 kcal · kg $^{-1}$; or *one of two milk-based beverages*, MILK 1: 5 kcal · kg $^{-1}$, 0.92 g · kg $^{-1}$ CHO, 0.21 g · kg $^{-1}$ Pro, 0.06 g · kg $^{-1}$ Fat; MILK 2: 5 kcal · kg $^{-1}$, 0.875 g · kg $^{-1}$ CHO, 0.375 g · kg $^{-1}$ Pro). Blood samples were collected prior to and immediately after the resistance exercise bout and at 60 min after consumption of the beverage. Vastus lateralis muscle samples were collected prior to exercise and 60 min after ingestion of beverage. **Results:** No significant differences were found among groups in MPS. The resistance exercise bout had an effect on muscle protein translational factors, increasing the amount of the active eIF4E•eIF4G complex by 4.5% 1-hr postexercise ($p<0.05$). No change was seen in the amount of eIF4E bound to 4E-BP1. One hour after beverage consumption, serum total amino acid concentration increased for MILK 1 ($p=0.003$) and MILK 2 ($p<0.001$), but decreased for CE ($p=0.028$) and PL ($p=0.276$). Consumption of MILK 1, MILK 2, and CE significantly increased circulating levels of serum insulin ($p<0.001$). Serum growth hormone increased 3-fold as a result of the exercise bout but fell to baseline for all groups by 60 min ($p<0.001$). **Conclusion:** The resistance exercise bout was anabolic as shown by the increase in the active eIF4E•eIF4G complex and in circulating growth hormone. Consumption of MILK 2 lead to the most optimal environment for muscle anabolism; however, none of the experimental beverages influenced the measured indicators of

muscle protein translation 1-hr after ingestion. **Keywords:** MUSCLE PROTEIN SYNTHESIS, MUSCLE PROTEIN DEGRADATION, EUKARYOTIC INITIATION FACTORS, INSULIN, GROWTH HORMONE, CARBOHYDRATE, PROTEIN

Introduction

The ideal body for a male in today's society consists of high muscle mass and low percentage of body fat, increasing the popularity of resistance weight training. Individuals performing weight training look for nutritional ways to accelerate gains in lean body mass without the use of expensive supplements.

Muscle accretion can only occur if muscle protein synthesis (MPS) is greater than muscle protein degradation (MPD). Resistance training has been shown to have a dramatic effect on protein metabolism by 50-112% increases in MPS (9, 10, 18, 44, 72) and 31-51% increases in MPD (9, 10, 44). However in the fasted condition, even with the dramatic increase in MPS, the rate of MPD exceeds the rate of synthesis, keeping the net muscle protein balance negative (9, 10, 44, 45, 61). Serum amino acids (2, 3, 11, 50, 59 – 63) and increased concentrations of some hormones (17, 23, 40, 55, 59) also stimulate muscle protein synthesis. Recent research has focused on the combination of resistance exercise with nutrient consumption designed to raise serum amino acids and anabolic hormones (11, 13, 41, 43, 50, 55, 60–63).

Biolo et al. (11) found that supplying amino acids via infusion 1-hr after exercise prevented the increase in MPD that normally ensues following resistance exercise. The proposed mechanism for this inhibition is decreasing the stimulus for MPD by maintaining the cellular uptake and intracellular concentration of amino acids above basal levels. Other researchers have suggested that supplying amino acids will promote MPS without increasing MPD over the resting rates (50, 59, 62). Even single amino acids have been shown to increase MPS (2, 3).

It is well established that certain hormones exert an anabolic effect on protein metabolism in humans. Human growth hormone (GH) is considered essential for skeletal muscle growth, acting through somatomedin activity to regulate growth and metabolism in humans (36). Human GH has a role in promoting MPS, assisting transport amino acids across the cell membrane, increasing RNA synthesis, promoting lipolysis, and promoting tissue growth via nitrogen retention (19, 35). Resistance exercise has been repeatedly shown to immediately increase serum GH (28, 35, 37–39, 58, 69). Researchers have also shown that supplementation with certain amino acids can influence GH concentrations (17, 58). Still to be determined is what nutritional supplementation immediately following resistance exercise would yield optimal concentrations of this hormone.

Insulin is another hormone that has been proposed to exert an anabolic effect on protein metabolism in humans. However, the role of insulin in muscle protein metabolism is still somewhat controversial. Some believe it is an important mediator of skeletal muscle anabolism, playing a role in both increasing MPS and attenuating MPD (8, 27, 34, 59). Biolo et al. (12) found that an insulin infusion increased MPS at rest, but not after exercise. However, Fluckey et al. (27) and Kimball et al. (34) found it essential after exercise for muscle protein anabolism. The consumption of carbohydrate or a combination of carbohydrate and protein after resistance exercise significantly increased plasma insulin levels (17, 50, 53, 54, 74). Muscle protein anabolism appears to be promoted when there is both an increase in availability of amino acids and elevated blood insulin concentration (8, 11, 59, 68). Rasmussen et al. (50) also examined MPS, finding a significant increase with the beverages that raised insulin levels. If the suggested roles of insulin are valid, what appears to be the most suitable environment for skeletal muscle accretion is an elevated concentration of serum insulin in the presence of sufficient amino

acids. Therefore, a nutritional intervention that increases both insulin and amino acids should produce the ideal environment for increasing MPS (27, 34).

There are still questions about composition of nutrient intake relative to resistance exercise for optimal MPS and MPD. The present study was designed to examine whether the consumption of several beverages, one containing only carbohydrate and electrolytes and two with both carbohydrate and protein, immediately following an acute resistance-exercise bout differentially influenced MPS and MPD.

Methods

Subjects. Forty healthy, but untrained males, age 18 to 25, volunteered to participate in this study that was approved by the Institutional Review Board for Research Involving Human Subjects of Virginia Tech, Blacksburg, Virginia. Subjects gave their informed consent and were screened for contraindications to participation in this study such as lactose intolerance, diabetes, cardiovascular diseases, orthopedic conditions, and allergies to lidocaine. All subjects had not performed resistance exercise for a minimum of 3 months. Subjects were randomly assigned to four groups (n=10): carbohydrate electrolyte beverage (CE), low-fat chocolate milk (MILK 1), high protein chocolate milk (MILK 2), and placebo (PL). After being accepted into the study, subjects underwent baseline testing to determine their maximal strength for leg extension and leg press (1RM). Subjects' 1RM was determined by increasing resistance for the exercise until the subject could only perform one repetition. Leg extension was performed using only the dominant leg for each subject. Subject characteristics can be found in Table 1.

Experimental Design. The subjects were given instructions on keeping a diet record, eating a meat-free diet, and 24-hr urine collection. One week prior to testing, the subjects completed a 3-day diet record. The subjects consumed a meat-free diet on the day before and the day of testing. Subjects arrived at the muscle-testing laboratory in War Memorial

Hall the morning of testing in a fasted condition, where they first had blood and muscle samples taken. After the first samples were taken, the subjects performed the acute exercise bout, consisting of five sets of eight repetitions of leg press and leg extension at 80% of 1RM with 2 min between sets and 4 min between exercises. After completing the last set, another blood sample was taken, and then subjects immediately consumed the provided beverage. One hour after the subjects consumed the test beverage, the final blood and muscle samples were taken. Twenty-four hour urine samples were collected the day before and the day of the resistance exercise bout. Immediately after and 1-hr after beverage consumption, subjects completed a Likert sensory form, evaluating the test beverage. An average of their two scores was used for sensory analysis of the beverages.

Post-Exercise Beverages. The subjects received either a non-caloric placebo beverage or an experimental beverage consisting of 5 kcal · kg $^{-1}$ after their resistance exercise bout. The PL was a flavored non-caloric beverage (Kraft Foods, Northfield, IL). CE was 1.25 g · kg $^{-1}$ of carbohydrate with electrolytes (Gatorade, Barrington, IL). MILK 1 was low-fat chocolate milk (Kroger, Cincinnati, OH), 0.92 g · kg $^{-1}$ carbohydrate, 0.21 g · kg $^{-1}$ protein, and 0.06 g · kg $^{-1}$ fat. MILK 2 was nonfat milk flavored with chocolate mix and fortified with instant nonfat dry milk (Kroger, Cincinnati, OH) to be similar to MILK 1 in volume, 0.875 g · kg $^{-1}$ carbohydrate and 0.375 g · kg $^{-1}$ protein.

Muscle Samples. Two biopsies of the vastus lateralis were obtained from all subjects under local anesthesia (lidocaine) using the percutaneous needle muscle biopsy technique with suction. To maximize sample size, a double pass was performed. The first biopsy was performed prior to exercise and the second an hour after the postexercise beverage was consumed. The biopsies were performed on the dominant leg, which was used for leg extension, approximately 2 cm from each other. The sample was immediately frozen in liquid nitrogen and stored in a freezer with a temperature of -80°C.

Using an anti-eIF4E monoclonal antibody, eIF4E was isolated by immunoprecipitation. The protein was electrophoretically transferred to a polyvinylidene difluoride membrane and incubated with polyclonal antipeptide antibody for eIF4E•eIF4G complex or eIF4E•4E-BP1 complex. The blots were then developed using Western blot technique (29).

Urine Samples. Subjects collected all of their urine excreted for 2 days in polypropylene bottles containing 1 mL 50% hydrochloric acid (the day before and the day of exercise testing). Collections began with the second void of the day and were turned in the following morning. Total volume was recorded and two 50-mL aliquots, for urinary 3MH and creatinine, were frozen at -20°C. Urinary 3MH was measured using the Waters Pico•Tag amino acid analyzer. Urinary creatinine was analyzed via spectrophotometery performing a manual assay with a commercially available kit (Sigma #555). All samples were analyzed in duplicate.

Blood Samples. A 15-mL blood sample was taken before the exercise bout, immediately postexercise, and 1-hr postexercise. After clot formation, samples were centrifuged at 3,000 rpm for 15 min at 4°C. Two milliliters of serum was diluted with a 1:2 dilution of methanol containing norleucine (NLE) as an internal standard for amino acid analysis. These samples were stored at -20°C until analysis. Serum glucose was analyzed spectrophotometrically using manual assay procedures from a commercially available kit (Kit #315). Serum insulin and growth hormone (GH) were analyzed by radioimmunoassay using commercially available kits (Diagnostic Products Corp., Los Angeles, CA).

Statistical Analysis. The main effect of group, time, and the interaction of groups by time for urinary 3MH, eIF, serum glucose, and hormones were analyzed by a two-way ANOVA with repeated measures (Sigma Stat 2.03, SPSS Inc.). Tukey's Post Hoc

analysis was used when a significant F-ratio was calculated. The main effect of group for subject baseline characteristics and Likert sensory data was analyzed by a one-way ANOVA (Sigma Stat 2.03, SPSS Inc.). Significance was defined as $p<0.05$.

Results

Anthropometrics. Subject characteristics are shown in Table 1. There were no significant differences between groups for age, height, weight, or body fat percentage. BMI for subjects in the CE group was significantly lower than subjects in MILK 1 ($p<0.05$). Since the calories given were based on the subject's weight, differences in BMI should not affect the results of other measurements.

Compliance. All subjects were questioned concerning compliance to urine collection and dietary instructions before performance trials. Three subjects reported deviance. Two subjects reported the consumption of some meat and the third forgot to collect urine. The urinary results were discarded for these three subjects.

Serum Analyses. Immediately postexercise and 1-hr postexercise, serum glucose concentrations were not significantly different from baseline in any of the groups ($p=0.208$) (Table 2). There was a statistically significant interaction between beverage group and time for serum insulin ($p<0.001$). One hour after consumption, there was a 3.6-fold increase in serum insulin for MILK 1, a 2.3-fold increase for MILK 2, a 2.8-fold increase for CE ($p<0.001$), and no change for Pl (Table 3). One hour after consumption of MILK 1, MILK 2, and CE, serum insulin was significantly greater than when the Placebo beverage was consumed ($p<0.001$).

Serum GH increased 275% immediately following exercise prior to beverage consumption ($p< 0.001$) (Table 4). One hour after the beverages were consumed, serum GH concentrations decreased similarly for all groups to preexercise concentrations.

A statistically significant interaction between beverage group and time was seen for serum total amino acids, EAA, and BCAA ($p<0.001$) (Table 5, Figure 2, Figure 3, & Figure 4). One hour after drinking MILK 1, concentrations of serum total amino acids ($p<0.01$) and EAA ($p<0.05$) were increased (10%, 8%) and a 9% increase in BCAA approached significance ($p=0.085$). Consumption of MILK 2 led to an increase in total amino acids (16%, $p<0.001$), EAA (14%, $p<0.001$), and BCAA (13%, $p<0.05$). However, the differences seen between MILK 1 and MILK 2 are not statistically significant (total AA: $p=0.990$; EAA: $p=0.852$; BCAA: $p=0.920$). The intake of CE following resistance training led to a decrease in total amino acids (7%, $p<0.001$), EAA (17%, $p<0.001$), and BCAA (26%, $p<0.05$). Placebo consumption after resistance exercise also lead to a decrease in EAA (9%, $p<0.05$) and BCAA (18%, $p<0.01$).

Urine Analyses. Beverage consumption had no statistically significant effect on MPD as indicated by urinary 3MH. There was an effect of resistance exercise on MPD in that urinary 3MH decreased by 54.1% the day of exercise compared to baseline ($p<0.05$) (Table 6). When examining the results of six vegetarian subjects, resistance exercise had no effect on MPD as indicated by urinary 3MH (Table 7).

Muscle Analyses. Beverage consumption had no statistically significant effect on translational factors. There was an effect of resistance exercise on muscle translational factors in that the active eIF4E•eIF4G complex increased 4.5% at 60 min postexercise ($p<0.05$) (Table 8). There was no change in amount of eIF4E bound to 4E-BP1 as a result of exercise or beverage ingestion (Table 9).

Sensory Analyses. Results are seen in Table 10. Using a Likert sensory form, subjects rated the taste of MILK 1 and MILK 2 significantly better than both CE and the placebo ($p<0.05$). No statistically significant difference was seen in taste between MILK 1 and MILK 2 or between CE and the placebo. Subjects rated MILK 2 more energizing

than MILK 1 ($p<0.05$). There were no significant differences between the beverages for quenching thirst or fulfilling hunger. When examining the mean score of all four categories combined, MILK 2 was rated significantly higher than the other three beverages ($p<0.001$) (Table 11).

Discussion

The authors of this study examined the effect of postexercise beverages differing in macronutrient concentrations on metabolite concentration and hormonal responses related to MPS and MPD following resistance exercise. The results indicate that providing a postexercise supplement with protein increases amino acids and insulin, while a carbohydrate supplement decreases amino acids concurrent with an increase in insulin. However, neither the measurement of muscle eukaryotic initiation factors associated with MPS in rodents nor MPD as measured by urinary 3MH were influenced by beverage consumption.

Amino acids have many physiological functions within the human body. Besides acting as precursors to protein, they play a regulatory role in both protein synthesis and protein degradation. In the present study, serum amino acid samples were collected preexercise and 1-hr postexercise. The changes seen in amino acid concentrations 1-hr postexercise are secondary to a combination of the resistance exercise and treatment beverages. By failing to measure serum amino acid concentrations immediately postexercise, we were unable to see any changes that may have resulted solely from exercise. Ideally, a sample should have also been collected immediately postexercise. Consumption of both milk-based beverages in the current study increased serum amino acids, while consumption of beverages without protein decreased serum amino acids (Figures 2-4). Vary et al. (66) found that increasing the concentration of plasma amino acids to 10-fold physiological levels stimulated protein synthesis by enhancing the

binding of eIF4E to eIF4G, thereby promoting translation initiation. However, it appears unnecessary to increase amino acid concentration to this extent. Using an infusion to increase the concentration of amino acids by approximately 70%, Rennie et al. (51) found the MPS rate had risen four times higher than the resting value within 2 hr. Rennie et al. (51) found that half maximal stimulatory concentration of amino acids was approximately 60% above the normal postabsorptive concentration and that very high plasma concentrations of amino acids, >2.5-fold normal, were required to saturate the system. The largest increase in total amino acids seen in the current study was 16%. It is possible that this modest increase in serum amino acids explains the lack of difference in effect on MPS between the experimental beverages.

It appears that it is not necessary to have an increase in every amino acid to stimulate skeletal MPS. Wolfe (70) speculated that it is plasma concentrations of EAA that regulate muscle protein metabolism in humans. Smith et al. (56) demonstrated that essential amino acids phenylalanine and threonine increase the incorporation of [^{13}C] leucine tracer amino acids into human skeletal muscle protein. Others have also shown that NEAA are unnecessary to stimulate skeletal MPS (13, 43, 50, 60–62). Two studies also demonstrated that intake of a large quantity of amino acids is unnecessary (13, 50). The ingestion of a relatively small amount (6 g) of EAA effectively stimulated net muscle protein balance after resistance exercise (13, 50). Individual plasma EAA increased by 75-150%, except for isoleucine and leucine which increased by 317% and 212% (13). When protein was provided in our study, individual serum EAA increased by 2-27% with isoleucine and leucine increasing by 21% and 15%. The largest increase in combined serum EAA was 14% in MILK 2. As suggested with the increase seen in total serum amino acids, the moderate increase in serum EAA could also possibly explain the lack of difference in effect on MPS between the experimental beverages.

There are some that believe that only BCAA are necessary for stimulation of MPS to occur. BCAA have been studied extensively to determine their effect on MPS. Vary et al. (66) concluded that the BCAA have a unique role in the process of increasing MPS after finding that a mixture of BCAA can support MPS as well as the full complement of amino acids. During the current study, BCAA represented 23% of the total protein given in the two milk-based postexercise beverages. In some studies performed at the University of Texas Medical Branch in Galveston, Texas, which showed increases in MPS after amino acid supplementation, 40% of the amino acids given were BCAA (13, 50, 60, 62). Of the BCAA, leucine appears to play an important role in regulating MPS. Anthony et al. (2, 4) found leucine was the only amino acid to stimulate translation initiation in the absence of other amino acids. Administration of leucine was the most effective in reducing the amount of the inactive eIF4E•4E-BP1 complex, thus increasing the availability of eIF4E for binding eIF4G, while neither valine nor isoleucine affected rates of MPS. Anthony et al. (1–3) suggested that the stimulatory effects seen after consumption of a protein-containing meal on translation initiation may be modulated in part by dietary leucine enhancing the formation of the active eIF4E•eIF4G complex. After feeding leucine, there is hyperphosphorylation of 4E-BP1, reducing its association with eIF4E, thus enhancing the formation of eIF4E•eIF4G (1–3, 33). Biolo et al. (11) also demonstrated leucine's ability to independently stimulate the recovery of MPS after exhaustive exercise. The deprivation of leucine causes the formation of eIF4E•eIF4G to become rate limiting (66). When leucine is removed from the medium, the amount of eIF4G that was immunoprecipitated with eIF4E was reduced by 80% and the amount of 4E-BP1 increased by 3.6-fold.

The previous studies described suggest that leucine would enhance the capacity of skeletal muscle to synthesize protein. Anthony et al. (2, 4) showed a 16-fold increase in

serum leucine when a leucine-containing meal was given to food-deprived rats and other studies (11, 13) showed approximately a 200% increase in plasma leucine when amino acids were provided postexercise to human subjects also in the postabsorptive state.. During the current study, only MILK 2 significantly increased serum leucine by 15% ($p<0.05$). Though statistically significant, the less marked increase in serum leucine in our study could potentially explain the lack of difference in effect on MPS between the experimental beverages.

A major difference in our study from previous studies is the use of whole protein instead of free amino acids. Acquiring amino acids from a whole protein source is more practical than using free amino acids. Milk is considered to be an excellent source of high quality protein, containing all of the essential amino acids. In contrast to the rapid absorption and delivery after ingestion of free amino acids, intact proteins are digested at variable rates, a factor that appears to independently regulate postprandial protein gain. Whey proteins, for example, are rapidly digested and stimulate amino acid oxidation and protein synthesis but have minimal or no effect on proteolysis (20). In contrast, casein is more slowly absorbed and consequently inhibits breakdown while increasing amino acid oxidation and protein synthesis to a lesser extent (20). This combination of proteins makes milk an obvious choice to use as an experimental beverage.

Since Tipton et al. (61) demonstrated that net protein balance is stimulated with postexercise supplementation of 40g amino acids, we examined both low fat milk and a milk beverage fortified with dry powdered milk. For our average subject of 76.6 kg, the quantity of protein ingested was 16 g and 29 g. In agreement with Borsheim et al. (13) and Rasmussen et al. (50), the extra protein had no additional stimulatory effect on MPS. In contrast with the previous studies, when subjects were provided protein, there was only a modest increase in serum amino acids and no effect of supplement intake on MPS.

This implies that with the differences in digestion and absorption, consumption of a greater quantity of whole protein may be necessary to increase serum amino acids to the same extent as free amino acids. Another possible implication is using one protein, such as whey, could have a greater effect on serum amino acids than milk, which is approximately 82% casein and 18% whey. Another area of further research could be looking at supplemention with a whole protein source at multiple time points, similar to that of Borsheim et al. (13) and Miller et al. (43).

The results from the present study showed decreases in urinary 3MH from baseline to the day of exercise suggesting that MPD is reduced on the day of resistance exercise. This is in contrast to other studies demonstrating either no change or an increase in urinary 3MH following exercise (14, 31, 32, 47, 53, 67). In our present study, the postexercise beverages had no effect on the reduction of urinary 3MH. Roy et al. (55) found a reduction in urinary 3MH when carbohydrates were given after an exercise bout versus a placebo. However, a second study performed by Roy et al. (53) examining what effect the macronutrient composition of beverages had on muscle anabolism and catabolism following resistance exercise found that 3MH excretion was not significantly different between any of the groups. These results conflict with their previous study (55), but support the results of our study. One drawback of the two previously mentioned studies (53, 55), is the lack of baseline 3MH values. This makes it difficult to interpret what effect resistance exercise has on 3MH excretion. Interpretation of our urinary 3MH data may be compromised by the fact that subjects only ate a meat-free diet on the days of collection. Some studies show that meat-free diets are necessary for 2-7 days prior to and during days of urine collection (6, 14, 21, 32, 42, 49). However, arguing against the need for prolonged meat-free diet, Pivarnik et al. (47) examined 3MH excretion every day during both the washout and experimental periods, finding no significant differences

in urinary 3MH for any day during the washout period. More evidence that a meat-containing diet did not affect our results is provided by examining the subset of vegetarian subjects. In the present study, six of the subjects were vegetarians and therefore did not need a washout period. The results from those six subjects can be seen in Table 6. 3MH also tended to decrease for these subjects after exercise, supporting the results of Radha and Bessman (49) which showed a 10-20% reduction in 3MH excretion after 1-hr of aerobic exercise. Although this suggests a washout period is not required, future studies should utilize a meat-free diet for a minimum of 1 day prior to measuring 3MH in order to exclude possible confounding by diet.

Not all research examining MPD analyzed 3MH excretion. Studies performed at the University of Texas Medical Branch in Galveston, Texas used isotopic tracers of amino acids to calculate MPD (9–12, 44, 45, 50, 60–63). The premise behind using isotopic tracers to calculate MPD is that phenylalanine is neither synthesized nor metabolized by skeletal muscle; therefore, the appearance rate of unlabeled phenylalanine reflects MPD (41). Phillips et al. (44) suggests that using isotopic tracers to calculated MPD is a more sensitive test than the use of 24-hr urinary 3MH excretion. Phillips et al. (44) found a 31% increase in MPD following resistance exercise via isotopic tracer methodology, but found no significant change in urinary 3MH. A trend toward increase in urinary 3MH was seen 48 hr postexercise ($p=0.084$) (44). Combining two studies, Biolo et al. (10) found that the 51% increase in MPD by resistance exercise was prevented by an infusion of amino acids administered 1-hr after exercise (11). However, the value of amino acids on MPD is controversial as a later study by the same laboratory found no change in MPD following resistance exercise (62). Our results supported two other studies that found neither the oral consumption of amino acids nor the combination of amino acids and carbohydrate after resistance exercise affected MPD compared to exercise alone (50, 61).

Though the use of isotopic tracers of amino acids to calculate MPD appears to be more sensitive than the use of urinary 3MH, there is no guarantee of its accuracy. It is possible that tissue other than skeletal muscle may significantly contribute to the appearance of the unlabeled amino acid, thus artificially increasing MPD. Currently, there is no evidence that the fluctuation in appearance of this amino acid from other sources would be stable from day to day. However, there has also been some controversy over the validity of 3MH as an indicator of MPD. Some researchers hold the opinion that urinary 3MH is not a good indicator of muscle damage from resistance exercise (31, 32, 52), while others support the validity of 3MH (6, 47, 49, 67). The most common criticism was that 3MH excretion was not coming from MPD alone, but also from other sources such as the gastrointestinal tract. Long et al. (42) examined this criticism, finding that the contribution of the small intestine appeared to be negligible, stating that urinary 3MH should continue to be a valid indicator of skeletal MPD. Another point to support the continued use of urinary 3MH is the fact that 3MH excretion from sources other than skeletal muscle remains stable from day to day. Therefore, any changes in 3MH excretion after exercise in our study would then solely be due to changes in MPD. Future research should use both urinary 3MH and isotopic tracer methodology to acquire a more complete picture of MPD dynamics.

Numerous studies using rodents have suggested that MPS is regulated by the availability of eIF4E to form the active eIF4E•eIF4G complex (1–4, 29, 33, 34, 66, 73). Decreased eIF4E availability occurs when 4E-BP1 sequesters eIF4E into an inactive complex, preventing eIF4G binding eIF4E (29). The acute exercise bout in the current study increased MPS as shown by a 4.5% increase in the eIF4E•eIF4G complex ($p<0.05$) without a change in the eIF4E•4E-BP1 complex. No effect on either eIF complexes was seen with postexercise supplementation. This suggests the acute resistance exercise bout

in the present study promotes translation initiation by augmenting the binding of eIF4E to eIF4G.

One hour following endurance exercise, rates of MPS were decreased in skeletal muscle of fasted rats (3, 29). When Gautsch et al. (29) fed rats a complete meal after exercise, eIF4E•eIF4G increased by approximately 380% compared to rats that were fasted after exercise. When compared to rats fed only carbohydrates after exercise, the amount of eIF4G associated with eIF4E was 3-fold greater in rats fed a complete meal. However, there was no difference in eIF4E•eIF4G between rats fed a complete meal after exercise and rats that did not exercise. Gautsch et al. (29) also found the amount of 4E-BP1 associated with eIF4E was approximately 4.6-fold higher in the rats that were fasted after exercise compared to the nonexercised controls. Providing food after exercise kept the quantity of eIF4E•4E-BP1 similar to the nonexercised control groups.

It is difficult to compare our results to those of Gautsch et al. (29) since our study involved resistance exercise as well as another species. However, it suggests that inclusion of protein in a postexercise meal is critical to stimulate change in translational factors related to MPS. For the present study, supplementation showed no effect on either the active eIF4E•eIF4G complex or the inactive eIF4E•4E-BP1 complex. There is one study that examined the eIF4E system in rats following resistance exercise, which reported a trend toward a greater amount of the active eIF4E•eIF4G complex without a change in the eIF4E•4E-BP1 complex (25). However, it is also difficult to compare the results of this study with our data since rates of MPS were not studied until 16 hr after the resistance exercise was completed and the rats were fed ad libitum during the first 9 hr after exercise.

One ribosomal protein kinase, S6K1, is known to play a role in regulating translation of mRNAs and translation elongation factors. Baar et al. (5) found phosphorylation of

S6K1 to be maximal 3-6 hr after exercise and is still more highly phosphorylated at 36 hr post-exercise than in control muscle. A positive relationship was also found between S6K1 phosphorylation and accumulation of muscle mass. Pitkanen et al. (46) found no changes in MPS or MPD 60 min after resistance exercise, but found an increase in both MPS and MPD 195 min after resistance exercise. Hernandez et al. (30) found that rates of MPS do not increase for at least 6 hr after acute resistance exercise. It is possible that an increase in the active eIF4E•eIF4G complex would have been seen if a muscle sample was taken at a later time, such as between 3 and 6 hr postexercise. One hour postexercise may have been too soon to see the changes in the binding states.

An alternative explanation is that our method to measure MPS may not be valid. To our knowledge, the present study was the first to examine changes in the active eIF4E•eIF4G complex to determine changes in MPS in human skeletal muscle. MPS could have increased significantly, but this method may not be able to detect the increase. It is possible that the key translational factors for MPS are different in human skeletal muscle than in rat skeletal muscle. Studies performed at the University of Texas Medical Branch in Galveston, Texas used isotopic tracers of amino acids to calculate MPS (9–12, 44, 45, 50, 60–63). Since phenylalanine is neither synthesized nor metabolized by skeletal muscle, the disappearance rate of labeled phenylalanine reflects MPS (41). This research group showed that the combination of resistance exercise and exogenous amino acids, given intravenously (11) or orally (13, 43, 50, 60, 62) stimulates MPS. MPS in these studies was increased 50% to 400% after administration of amino acids.

Future studies should incorporate both isotopic infusion methodology and the examination of changes in the active eIF4E•eIF4G complex as well as other translational factors to validate the later method of analyzing MPS in human skeletal muscle. Another potential area of future research is examining the role of tryptophan postexercise in it

relationship to MPS. Of the studies performed at the University of Texas Medical Branch in Galveston, Texas examining skeletal MPS, there were two instances where a significant increase in MPS was not seen with the combination of resistance exercise and oral doses of amino acids (61, 62). The most notable difference between the first study (61) and other studies from this lab (13, 43, 50, 60, 62) is the presence of tryptophan in the amino acid mixture (61). In both this study by Tipton et al. (61) and our current study using milk-based postexercise beverages, tryptophan represented 3% of the EAA given.

Tipton et al. (62) gave an oral dose of EAA with sucrose either immediately prior to or immediately following resistance exercise. When the supplement was given preexercise, a dramatic increase in MPS was seen. However, MPS was not significantly affected when the supplement was given immediately postexercise. This was the only study performed by this group of researchers which examined supplementation immediately postexercise. This further supports the need for more research examining multiple time points of whole protein supplementation in combination with resistance exercise.

The endocrine system nfluences growth and development by its vital role in the regulation of protein metabolism. Anabolic hormones, such as insulin and growth hormone, not only promote protein synthesis but can also aid in preventing degradation. Therefore, one key to promoting positive protein balance is finding the best approach to increasing the response of anabolic hormones after resistance exercise.

Insulin is believed to be closely related to muscle protein metabolism. All three experimental beverages in our study produced insulin levels significantly higher than baseline (Table 2). The role of insulin in muscle protein dynamics is still controversial. Some have found insulin necessary after exercise for MPS (7–9, 24, 26, 27, 51), while other researchers have found MPS after exercise was not dependent on circulating insulin

levels (2, 3, 11, 12). While a number of researchers examined insulin's role in MPS, others suggested that insulin also has the ability to suppress the increase in MPD after exercise (8, 10, 12, 59). However, in the current study, resistance exercise stimulated a decrease in MPD as indicated by a decrease in 3MH, and the rates of MPD were not different with differing serum insulin concentrations.

From previous research, it appears that rapid recovery of protein synthesis following exercise is most efficient with both elevated plasma insulin and dietary protein or amino acids. Preedy and Garlick (48) reported that infusion of glucose and amino acids stimulated protein synthesis in rats following 12-hr food deprivation. Conversely, infusion of amino acids and glucose plus insulin antiserum did not stimulate MPS rates despite a significant rise in plasma glucose concentrations. Similarly, Yoshizawa et al. (73) reported that refeeding mice a nutritionally complete meal, but not a protein-free meal, stimulated MPS following overnight food deprivation. These researchers concluded that amino acids and insulin act together to stimulate protein synthesis following a fast (48, 73). Rennie et al. (51) found that only a small increase in insulin is necessary for this stimulation and large increases have no further effect on MPS, supporting the theory that exercise generates a greater sensitivity to the events regulated by insulin (41).

Insulin response after supplement ingestion is controlled by the composition of the beverage. In some studies, the combination of carbohydrate and protein has been shown to cause a significant insulin rise beyond that seen for carbohydrate alone (17, 57, 65, 74). However, the energy content of the experimental beverages in those studies was often not the same. When the beverages were isoenergetic, there was no difference in insulin (16, 65). All three experimental beverages from the present study caused a significant rise in insulin concentration when compared to the placebo. MILK 1 showed

the greatest insulin response, 25% greater than CE and 50% greater than MILK 2; however, there was no significant difference between the three beverages. Our results support the previous findings that when the experimental beverages differing in carbohydrate/protein concentrations are isocaloric, there is no difference in insulin response (16, 65). In the present study, MPS was not significantly different between the four differing concentrations of insulin seen, supporting the theory that other factors play a greater role in promoting MPS than insulin concentration (2, 3, 11, 12).

Growth hormone is another anabolic hormone with a well-established role in increasing MPS. For over 60 years, GH has been known to be essential for normal growth of skeletal muscle (36). Other known roles of GH are decreasing glycogen synthesis, increasing availability of glucose and amino acids, and increasing the transport of amino acids across cell membranes. However, many physiological stimuli such as exercise, sleep, diet, and stress can stimulate GH release. During the present study, GH significantly increased immediately following resistance exercise, supporting previous research (35, 37–39). Similar to the results of Fogelholm et al. (28) and Suminski et al. (58), the postexercise increase in GH concentration was unchanged by the intake of the experimental beverages. Conversely, Chandler et al. (17) found supplementation can stimulate the release of GH and that supplements that cause the greatest insulin spike after exercise lead to the greatest GH levels 5-6 hr postexercise, possibly related to the insulin-induced hypoglycemia. The last data collection in the current study was 1-hr postexercise. Therefore it is possible that GH fluctuations between groups would have been observed 5-6 hr postexercise.

From a practical standpoint, it is important that a pre- or postexercise beverage be easy to obtain, relatively inexpensive, and palatable. The beverages used our study, or the components there of, can easily be purchased at a local grocery store. Each beverage

was a small fraction of the cost of amino acid supplements or commercial postexercise drinks. Subjects also rated both milk-based beverages highly on taste. Therefore, the milk-based drinks are very practical choices for postexercise beverages.

Summary

The resistance exercise bout increased the amount of eIF4G associated with eIF4E in human skeletal muscle and lead to a significant decrease in urinary 3MH excretion. However, the postexercise beverages did not affect the formation of the active eIF4E•eIF4G complex or the excretion of urinary 3MH. Some useful information was gained from this study. Both milk-based beverages were successful at increasing serum amino acids and insulin when given immediately following a resistance exercise bout. The consumption of a carbohydrate-electrolyte beverage after a resistance exercise bout increased circulating levels of insulin, but lead to a significant decrease in total amino acids, EAA, and BCAA. Drinking a non-caloric beverage postexercise also lead to a significant decrease in serum EAA and BCAA. This has important implications further supporting the need for a combination of protein and carbohydrates following resistance exercise. The lack of effect on skeletal muscle translational factors or urinary 3MH in spite of differences in serum amino acids among beverages suggests that additional research should be done to further examine the relationship among these factors.

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Table 1. Subject Characteristics

	Age (years)	Height (m)	Weight (kg)	BMI	Body Fat (%)
MILK 1	21.9 ± 0.55	1.76 ± 0.03	81.48 ± 4.22	26.10 ± 0.73 *	12.54 ± 2.9
MILK 2	21.8 ± 0.57	1.80 ± 0.02	75.66 ± 3.20	23.46 ± 0.82	11.73 ± 1.1
CE	20.2 ± 0.47	1.77 ± 0.03	69.45 ± 2.95	22.16 ± 0.62 *	10.39 ± 2.0
Placebo	21.1 ± 0.56	1.79 ± 0.02	80.00 ± 5.84	24.92 ± 1.38	14.14 ± 2.6

Values are expressed as mean ± SE. n=10.

* – Indicates significant difference between groups ($p<0.05$).

Table 2. Serum glucose concentration (mg/dL) before and after acute resistance exercise bout.

Group	Pre	IPE	Post
MILK 1	93.7 ± 6.65	91.85 ± 4.90	85.80 ± 9.98
MILK 2	85.80 ± 5.21	95.18 ± 5.74	78.76 ± 8.52
CE	93.09 ± 4.85	81.85 ± 5.04	85.80 ± 11.84
Placebo	92.22 ± 6.45	86.54 ± 5.26	85.56 ± 7.24

Values are expressed as mean ± SE. n=9.
Pre: PreExercise; IPE: Immediately PostExercise; Post: 1-Hr PostExercise.

Table 3. Insulin (μIU/mL) before and after acute resistance exercise bout.

Group	Pre	IPE	Post
MILK 1	17.02 ± 2.8	16.12 ± 1.8	74.04 ± 16.8 [ab]
MILK 2	13.16 ± 1.0	15.31 ± 2.1	51.27 ± 5.9 [ab]
CE	16.37 ± 1.7	14.92 ± 2.2	56.83 ± 11.4 [ab]
Placebo	17.61 ± 2.4	15.82 ± 1.8	16.66 ± 1.7

Values are expressed as mean ± SE. n=9.
Pre: PreExercise; IPE: Immediately PostExercise; Post: 1-Hr PostExercise.

a – Indicates significant difference from Pre and IPE within group ($p<0.05$)
b – Indicates significant difference from Placebo Post ($p<0.05$)

Table 4. Human growth hormone (ηg/mL) before and after acute resistance exercise bout.

Group	Pre	IPE *	Post
MILK 1	1.04 ± 0.03	2.93 ± 0.52	1.04 ± 0.03
MILK 2	1.00 ± 0.00	3.98 ± 1.00	1.01 ± 0.01
CE	1.26 ± 0.25	5.88 ± 1.37	1.02 ± 0.01
Placebo	1.03 ± 0.04	3.46 ± 0.65	1.00 ± 0.00

Values are expressed as mean ± SE. n=8.
Pre: PreExercise; IPE: Immediately PostExercise; Post: 1-Hr PostExercise.

* – Indicates significant difference between this time point and all others ($p<0.05$)

Table 5. Serum amino acids before and after acute resistance exercise bout.

	Total AA (mg/L)		EAA (mg/L)		BCAA (mg/L)	
Group	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
MILK 1	422.9 ± 13.3	464.1 ± 16.5 *	166.7 ± 5.9	180.7 ± 6.2 *	63.5 ± 3.8	69.1 ± 3.6
MILK 2	404.7 ± 8.7	469.6 ± 8.3 *	152.9 ± 3.9	174.8 ± 6.0 *	58.7 ± 1.4	66.6 ± 3.4 *
CE	386.9 ± 9.3	357.9 ± 16.0 *	142.5 ± 5.5	118.5 ± 4.5 *	53.7 ± 2.4	39.7 ± 2.5 *
Placebo	403.5 ± 12.3	388.7 ± 16.2	153.4 ± 3.7	138.9 ± 5.7 *	59.8 ± 2.2	49.1 ± 2.8 *

Values are expressed as mean ± SE. n=10.
Pre: PreExercise; Post: 1-Hr PostExercise.

* – Indicates significant difference between Pre and Post value ($p<0.05$).

Table 6. Urinary 3MH/Creatinine Data (μmol/g) before and after exercise.

Group	Day 1	Day 2 *
MILK 1	226.34 ± 56.7	150.23 ± 58.4
MILK 2	298.21 ± 101.5	80.71 ± 19.7
CE	238.20 ± 90.8	102.06 ± 32.7
Placebo	400.92 ± 143.6	150.22 ± 39.8
Total	239.95 ± 45.5	110.03 ± 20.0

Values are expressed as mean ± SE. n=8
Total represents combination of all groups. n=32.

* – Indicates significant difference between this point and Day 1.

Table 7. Individual vegetarian subject 3MH/Creatinine Data before and after exercise.

Group	Subject	3MH/Creatinine (µmol/g)	
		Day 1	**Day 2**
MILK 2	28	36.494	21.194
MILK 2	42	31.639	32.363
CE	27	22.275	20.647
CE	37	34.442	29.017
Placebo	14	26.428	26.199
Placebo	40	39.229	34.693
	Mean	**31.751**	**27.352**
	± SEM	**2.605**	**2.352**

Table 8. eIF4G associated with eIF4E before and after acute resistance exercise bout.

Group	Pre	Post
MILK 1	176.68 ± 14.77	182.67 ± 15.30
MILK 2	193.13 ± 15.57	205.80 ± 11.19
CE	171.23 ± 16.55	176.44 ± 16.49
Placebo	181.50 ± 8.70	190.02 ± 6.75
Total	180.64 ± 6.96	188.73 ± 6.55 *

Values are expressed as mean ± SE. n=10.
Pre: PreExercise; Post: 1-Hr PostExercise.
Total represents combination of all groups. n=40.

* – Indicates significant difference between Pre and Post value.

Table 9. 4E-BP1 associated with eIF4E before and after acute resistance exercise bout.

Group	Pre	Post
MILK 1	163.85 ± 13.04	161.08 ± 11.40
MILK 2	188.21 ± 15.32	188.23 ± 13.92
CE	176.59 ± 19.85	167.81 ± 21.24
Placebo	160.95 ± 8.71	160.07 ± 9.62
Total	172.40 ± 7.31	169.30 ± 7.30

Values are expressed as mean ± SE. n=10.
Pre: PreExercise; Post: 1-Hr PostExercise.
Total represents combination of all groups. n=40.

Table 10. Likert analysis of postexercise beverages.

Group	Taste	Quenches Thirst	Fulfills Hunger	Energizing
MILK 1	11.438 ± 0.411 [a]	7.788 ± 0.679	8.525 ± 0.629	6.838 ± 0.664 [b]
MILK 2	11.713 ± 0.448 [a]	10.475 ± 0.402	10.100 ± 0.463	9.950 ± 0.496 [b]
CE	9.200 ± 0.991	9.500 ± 0.995	6.538 ± 0.938	8.250 ± 0.957
Placebo	8.513 ± 0.690	10.525 ± 0.607	7.213 ± 0.790	8.050 ± 0.740

Values are expressed as mean ± SE. n=10.
Scores range from 0 – 14.

a – Indicates significant difference from CE and Placebo ($p<0.05$)
b – Indicates significant difference between groups ($p<0.05$)

Table 11. Likert analysis of postexercise beverages: Combined score of all categories.

Group	Overall Score
MILK 1	8.647 ± 0.354
MILK 2	10.559 ± 0.236 *****
CE	8.372 ± 0.493
Placebo	8.575 ± 0.374

Values are expressed as mean ± SE. n=10.
Scores range from 0 – 14.

* – Indicates significant difference from all other points ($p<0.001$)

Figure 1. Timeline of procedures.

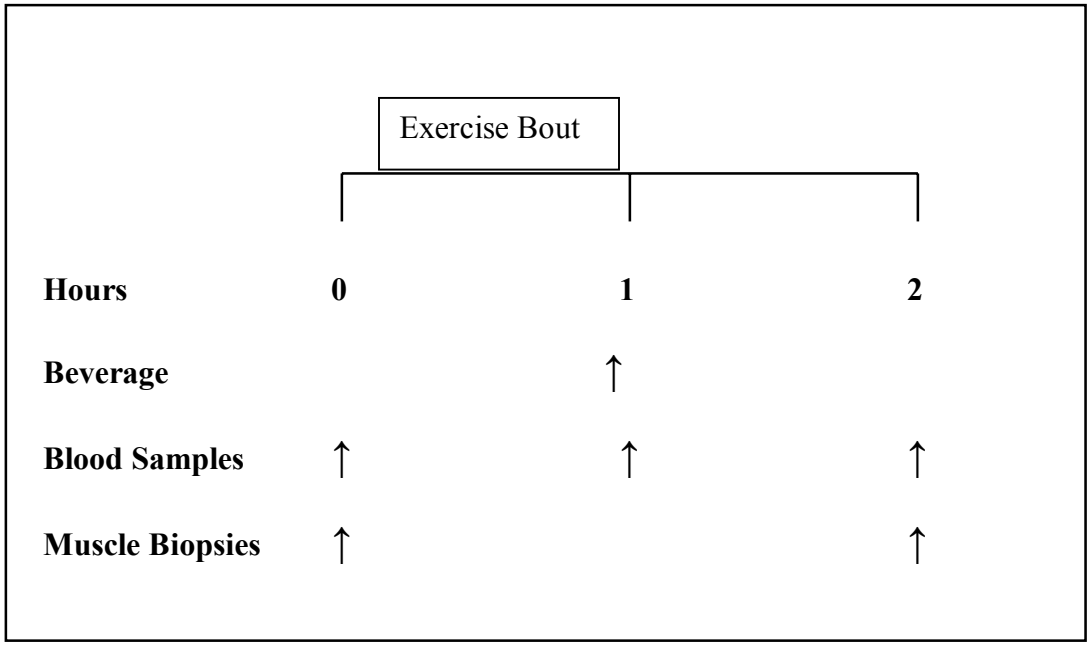
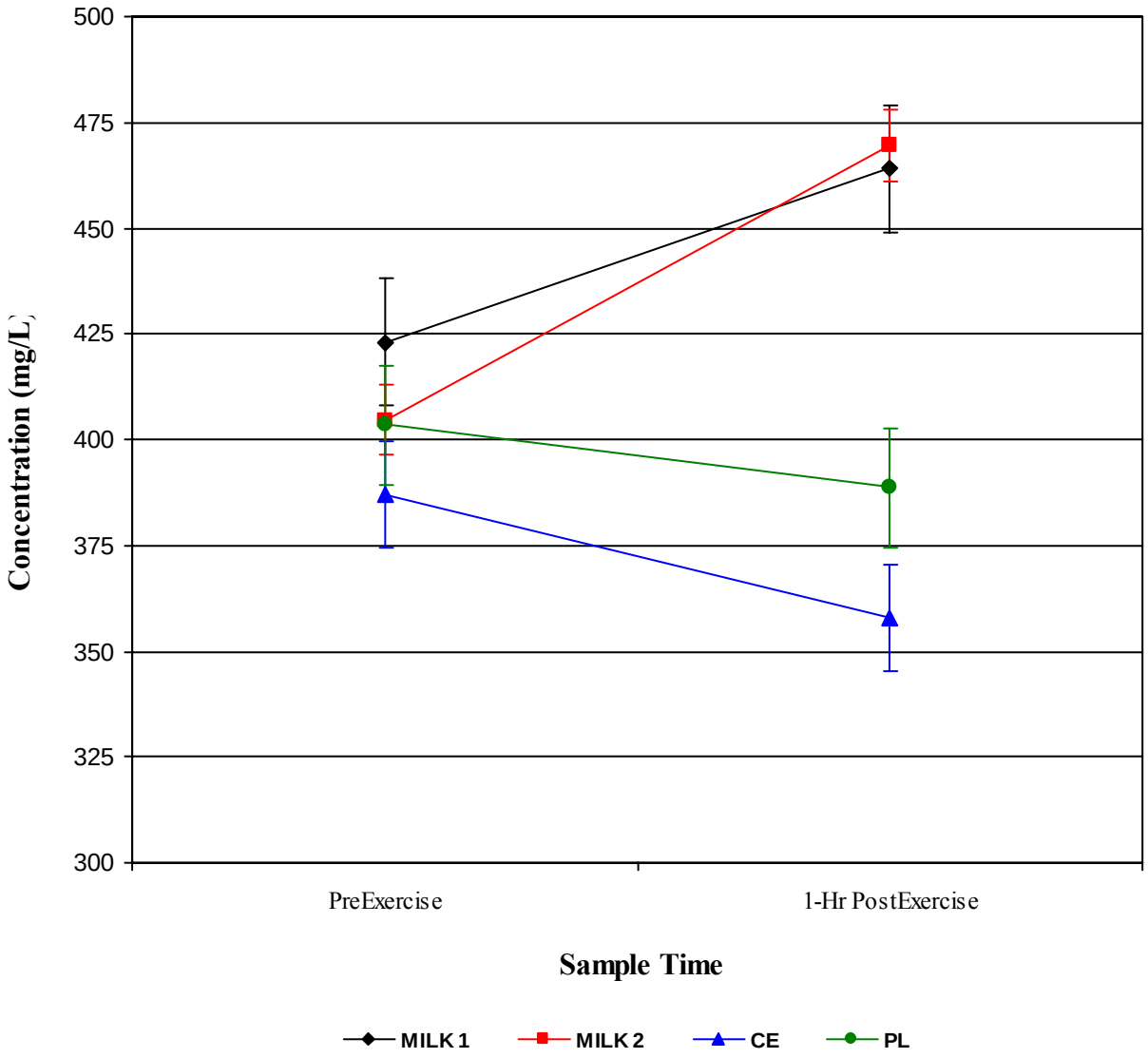
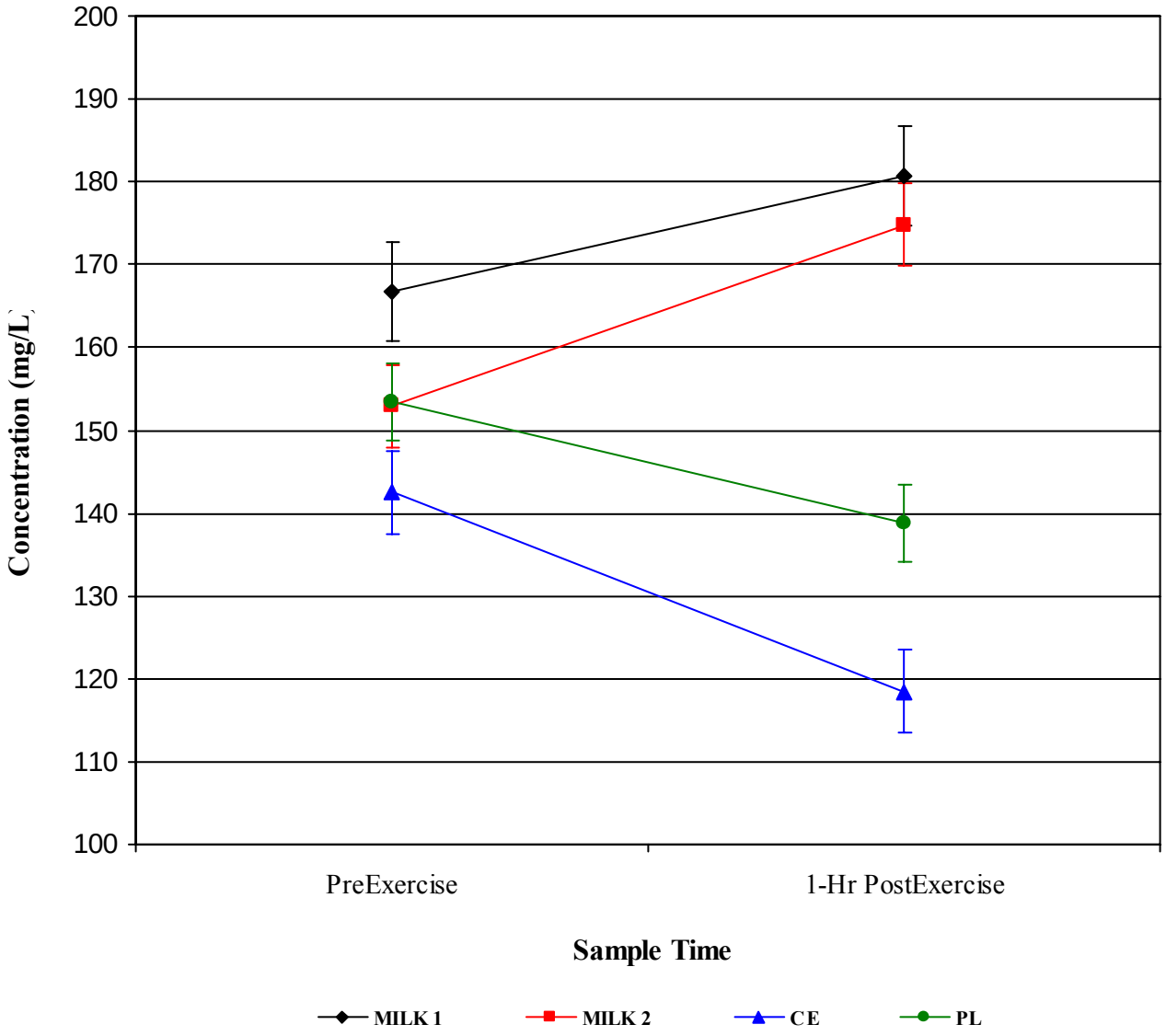


Figure 2. Concentration of serum total amino acids before and after exercise and supplementation.



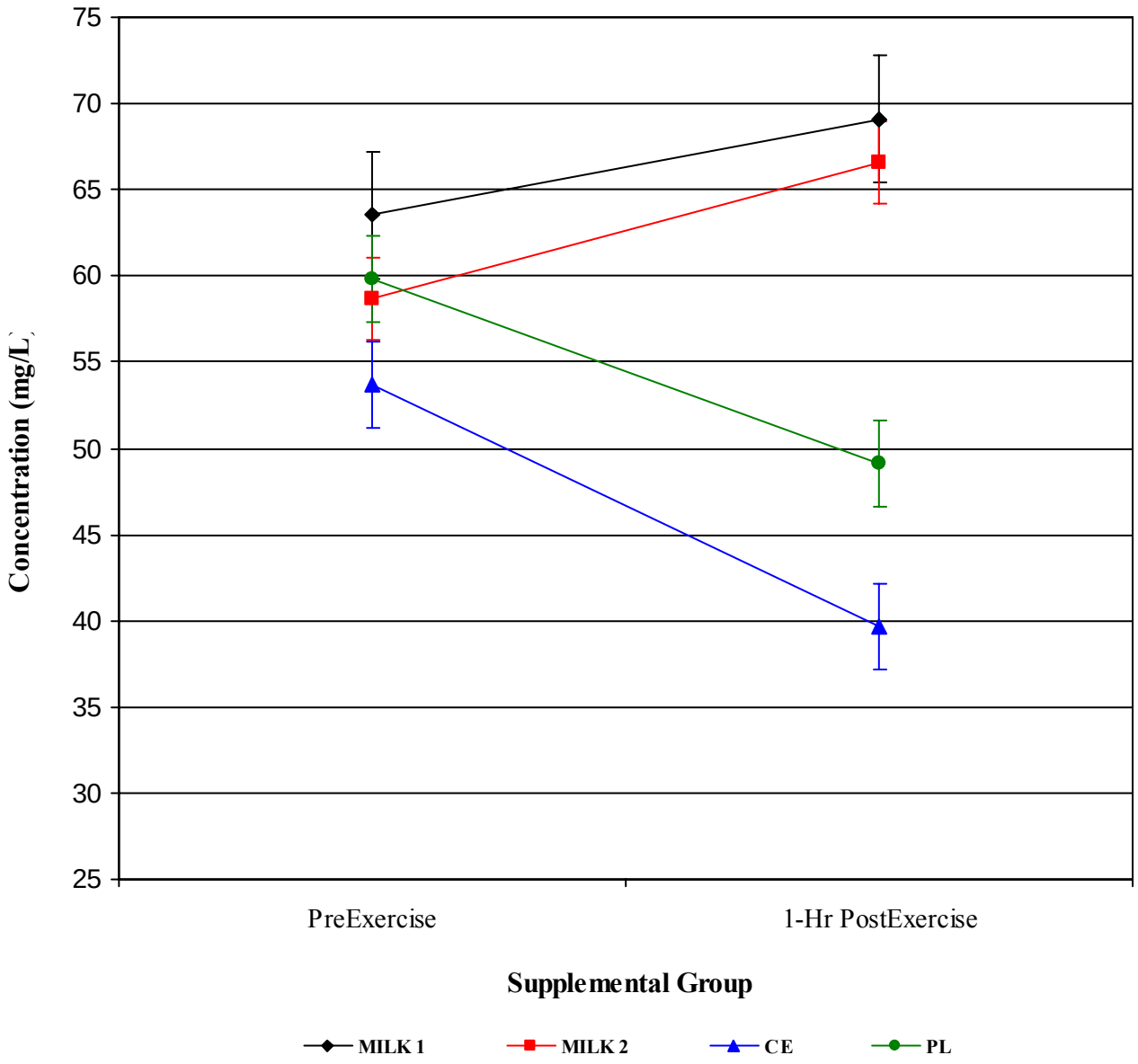
Concentration of serum Total AA is significantly different 1-Hr Postexercise vs. Preexercise for MILK 1, MILK 2, and CE ($p<0.05$).

Figure 3. Concentration of serum EAA before and after exercise and supplementation.



Concentration of serum EAA is significantly different 1-Hr Postexercise vs. Preexercise for all groups ($p<0.05$).

Figure 4. Concentration of serum BCAA before and after exercise and supplementation.



Concentration of serum BCAA is significantly different 1-Hr Postexercise vs. Preexercise for all groups ($p<0.05$).

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Chapter 4:

Summary and Recommendations

Postexercise supplementation is a widespread practice among people who perform resistance exercise on a regular basis. If there are marketing claims that a product will increase strength and muscle size, people will buy it. This is why nutritional supplements are such a big industry today.

The purpose of the present study was to determine the effect of four post-exercise beverages differing in macronutrient content on metabolic response to an acute resistance exercise bout. Forty healthy males age 18 to 25 who had not performed resistance training for a minimum of 3 months volunteered to participate in this study. Subjects were randomly assigned to four groups: CE (n=10), MILK 1 (n=10), MILK 2 (n=10), and PL (n=10). PL was a flavored non-caloric beverage (Kraft Foods, Northfield, IL). CE was 1.25 $g \cdot kg^{-1}$ of carbohydrate with electrolytes (Gatorade, Barrington, IL). MILK 1 was low-fat chocolate milk (Kroger, Cincinnati, OH), 0.92 $g \cdot kg^{-1}$ carbohydrate, 0.21 $g \cdot kg^{-1}$ protein, and 0.06 $g \cdot kg^{-1}$ fat. MILK 2 was nonfat milk flavored with chocolate mix and fortified with instant nonfat dry milk (Kroger, Cincinnati, OH) to be similar to MILK 1 in volume, 0.875 $g \cdot kg^{-1}$ carbohydrate and 0.375 $g \cdot kg^{-1}$ protein.

The subjects consumed a meat-free diet on the day before and the day of testing. Subjects arrived at the muscle-testing laboratory in a fasted condition. The exercise bout consisted of five sets of eight repetitions of leg press and leg extension at 80% of 1RM with 2 min between sets and 4 min between exercises. Twenty-four hour urine samples were collected for analysis of 3MH/Creatinine excretion; blood samples were collected for analysis of serum metabolites and hormones; and muscle samples were obtained for analysis of eIF.

The results from the present study showed decreases in urinary 3MH from baseline to the day of exercise. Interpretation of our urinary 3MH data may be compromised by the fact that subjects only ate a meat-free diet on the days of collection. Other studies show

that meat-free diets are necessary for 2-7 days prior to and during days of urine collection (Ballard and Tomas 1983, Calles-Escandon et al. 1984, Dohm et al. 1983, Horswill et al. 1988, Long et al. 1988, & Radha and Bessman 1983). Pivarnik et al. (1989) examined 3MH excretion every day during both the washout and experimental periods, finding no significant differences in urinary 3MH for any day during the washout period. So it is possible that the lack of a true washout period may not significantly affect our results. But there was a slight decrease from day 1 to day 2 followed by very consistent concentrations, indicating it would be best to use a meat-free diet for a minimum of 1 day prior to measuring 3MH. In the present study, six of the subjects were vegetarians and therefore did not need a washout period. The results from those six subjects can be seen in Table 6. 3MH also tended to decrease for these subjects after exercise, supporting the results of Radha and Bessman (1983) which showed a 10-20% reduction in 3MH excretion for each subject.

For MPS to occur, constituents such as amino acids need to be available. Biolo et al. (1997) found that during hyperaminoacidemia, protein synthesis was approximately 150% higher after exercise than rest, suggesting that anabolism will be greater if resistance exercise is paired with hyperaminoacidemia. Vary (1999) demonstrated that elevating amino acid concentration enhances the binding of eIF4E to eIF4G, thereby promoting translation initiation. Yoshizawa et al. (1998) found no change in MPS when rats were fed diets containing 0% protein and an increase of 38% in MPS was seen when rats were fed diets containing 20% protein. This increase was associated with reduced binding of 4E-BP1 with eIF4E and increased formation of eIF4E•eIF4G. Anthony et al. (2000a) also found that the amount of 4E-BP1 associated with eIF4E was unchanged when the rats were fed carbohydrate alone, but was significantly reduced with the administration of leucine or the combination of leucine and carbohydrate. Other studies

have also demonstrated that of all amino acids only EAA are necessary to increase MPS (Borsheim et al 2002, Rasmussen et al. 2000, Tipton et al. 1999, 2001, 2003). Tipton et al. (1999) showed that an oral dose of amino acids is just as effective as infusion at inducing hyperaminoacidemia. In the present study, only MILK 2 lead to significant increases in Total AA, EAA, BCAA, and leucine concentrations 1-hr postexercise. After consumption of MILK 1, Total AA and EAA increased significantly while a trend toward increasing was seen in BCAA ($p=0.085$). The opposite was seen when carbohydrate but not protein was provided following the resistance exercise bout. Consumption of CE lead to significant decreases in circulating levels of Total AA, EAA, BCAA, and leucine. Drinking the placebo beverage did not effect the concentration of Total AA, but did lead to a significant decrease in EAA, BCAA, and leucine. Of the experimental beverages, only MILK 2 increased circulating amino acids in a manner most likely to stimulate MPS.

The exercise bout performed was effective at increasing serum GH levels. Circulating levels of GH increased 275% immediately following exercise prior to beverage consumption ($p< 0.001$) (Table 3). One hour after the beverages were consumed, serum GH concentrations decreased to preexercise concentrations in all groups.

One hour after the resistance exercise bout, serum glucose concentrations were not significantly different from baseline in any of the groups. It is most likely that glucose concentrations peaked after the experimental beverages were consumed and returned to baseline prior to the last blood sample 1-hr postexercise. A peak and decline in serum glucose would explain the increase seen in circulating levels of insulin 1-hr postexercise.

When compared to consumption of the placebo, all three experimental beverages led to a significant increase in serum insulin concentration ($p<0.01$). Insulin has been suggested to be a mediator in both MPS and MPD. Kimball et al. (1997) found that

insulin promoted the phosphorylation of 4E-BP1, freeing eIF4E from the inactive eIF4E•4E-BP1 complex, thus allowing it to bind to eIF4G. Other previous research has demonstrated the need for insulin after resistance exercise to have elevated MPS rates (Anthony et al. 2002, Balage et al. 2001, Fluckey et al. 1996, Preedy & Garlick 1986, Sinaud et al. 1999, Svanberg et al. 1996a). Sinaud et al. (1999) also demonstrated that the lack of insulin inhibits MPS by reducing the assembly of the active eIF4F complex. In contrast, Biolo et al. (1999) found that insulin infusion increased protein synthesis at rest, but had no effect after exercise. The authors suggested that this difference might have been due to MPS being limited by amino acid availability. In addition, Biolo et al. (1999) demonstrated insulin's ability to suppress MPD is increased after resistance exercise.

Muscle protein anabolism appears to be promoted when there is an increase in both amino acid availability and circulating levels of insulin (Bennet et al. 1990, Svanberg et al. 1996a, Volpi et al. 1998). In concert with this statement, Rasmussen et al. (2000) concluded that the consumption of the combination of essential amino acids and carbohydrates promotes muscle anabolism. It appears that insulin and amino acids independently promote the phosphorylation of 4E-BP1, but are more effective when provided in combination (Kimball et al. 2002b).

Beverage consumption had no statistically significant effect on translational factors, even though serum amino acids and insulin were increased after drinking both milk-based beverages. There was an effect of resistance exercise on muscle translational factors in that the active eIF4E•eIF4G complex increased 4.5% at 60 min postexercise ($p<0.05$) (Table 7). There was no significant change in amount of eIF4E bound to 4E-BP1 as a result of exercise or beverage ingestion (Table 8).

Recommendations for Future Research

Some questions have risen or remain controversial as a result to the current study. Does the eIF4E system play the same role in human skeletal muscle as it does in rodents? Is the measurement of the active eIF4E•eIF4G complex a valid measure of MPS in humans? Another area that remains controversial is the effect of resistance exercise on MPD and the use of urinary 3MH as an indicator of MPD. More information could be obtained on these topics by performing a study similar to ours but adding the isotopic tracer methodology to also ascertain the validity of our MPS and MPD measurements. Timing of amino acid supplementation in relation to an exercise bout has been studied extensively. Similar studies are needed with whole protein sources. Also, can a whole protein source increase serum amino acids and MPS to the same extent as free amino acids?

1. Timing is critical for every part of this research study. Timing of the biopsy may determine whether a significant increase in MPS is seen. To determine increases in MPS, we examined the binding states of eIF4E, eIF4G, and 4E-BP1 in skeletal muscle. The binding of 4E-BP1 is regulated by phosphorylation of 4E-BP1. An increase in phosphorylation decreases its binding capacity and a decrease in phosphorylation promotes binding. Jefferson and Kimball (2001) stated that although the identity of the kinases(s) that phosphorylated 4E-BP1 is (are) still undefined, it is known that phosphorylation of 4E-BP1 can occur in response to either amino acids or anabolic hormones. The environment created after resistance training and milk consumption should therefore promote an increase in phosphorylated 4E-BP1. However, in our study, the beverages that provided amino acids did not affect the binding of 4E-BP1 more than the beverages that were void of amino acids. One

ribosomal protein kinase, S6K1, is known to play a role in regulating translation of mRNAs and translation elongation factors. Baar et al. (1999) found phosphorylation of S6K1 to be maximal 3-6 hr after exercise and is still more highly phosphorylated at 36 hr post-exercise than in control muscle. A positive relationship was also found between S6K1 phosphorylation and accumulation of muscle mass. Pitkanen et al. (2003) found no changes in MPS or MPD 60 min after resistance exercise, but found an increase in both MPS and MPD 195 min after resistance exercise. Hernandez et al. (2000) found that rates of MPS do not increase for at least 6 hr after acute resistance exercise. It is possible that an increase in the active eIF4E•eIF4G complex would have been seen if a muscle sample was taken at a later time, such as between 3 and 6 hr postexercise. One hour postexercise may have been too soon to see the changes in the binding states.

2. For this study, we used three different quantities of protein in the postexercise beverages, 0 $g \cdot kg^{-1}$, 0.21 $g \cdot kg^{-1}$, and 0.375 $g \cdot kg^{-1}$. For our average subject of 76.6 kg, this is 0 g, 16 g, and 29 g of protein. The exercise in our study elicited a 4.5% increase in eIF4E•eIF4G at 60 min postexercise, with no change in quantity of eIF4E•4E-BP1. The supplemental beverages had no effect on either complex. Volpi et al. (1998) found a significant increase in MPS using a total amino acid infusion of 148.5 $mg \cdot kg^{-1}$. A few studies demonstrated that the intake of 6 g of EAA promotes anabolism (Borsheim et al 2002, Rasmussen et al. 2000, Tipton et al. 2001). Our study is one of very few that used a whole protein source instead of amino acids. Yoshizawa et al. (1998) found an increase of 38% in MPS when rats were fed a meal consisting of 20% protein after they had been food deprived for 18 hr. However, these rats were fed ad libitum for 1-hr after exercise, consuming ~25% of a typical day's intake.

The estimated RDA is approximately 95.5 mg · kg $^{-1}$ EAA (Williams 1999). For our average subject of 76.6 kg, this is 7.3 g. Assuming that approximately 45% of milk protein is comprised of EAA, our two protein-containing beverages provided approximately 7g and 13g of EAA. Our milk-based beverages met or exceeded the RDA for EAA and also provided more EAA than others found to stimulate MPS (Borsheim et al 2002, Rasmussen et al. 2000, Tipton et al. 2001). It is possible that when ingesting whole protein, more is needed to elicit similar increases in serum amino acids and promote MPS. More research is needed to determine the quantity of EAA from a whole protein source necessary to elicit MPS.

3. For most studies looking at supplementation after resistance exercise, subjects all consumed the supplement at the same time within the study (Rasmussen 2000, Roy 1997, 1998, 2000, Suminski, Tarnopolsky 1997, Tipton 1999). Our study was no different. All subjects were given the supplement immediately after exercise. It would be beneficial for a study to examine multiple time points for the ingestions of the supplement, as to determine the optimal timing for ingestion.

4. There is controversy over how to measure MPD. Some agree with the use of urinary 3MH (Ballard and Tomas 1983, Bucciante et al. 1985, Calles-Escandon 1984, Dohm 1985, Long 1988), while others believe the best way is to measure the rate of appearance of labeled amino acids (Biolo 1992, 1995, 1997, 1999). We believe that both techniques are valid indicators of MPD, with measuring urinary 3MH being the safest and most economical. Future studies should incorporate both isotopic infusion methodology and the examination of changes in urinary 3MH. This could possibly eliminate the controversy involved in measuring MPD. The best measure of MPD would be an analysis that is less expensive than using isotope tracers; one that is not compromised by diet; and one that is still safe. We were the first to measure

initiation factors in human muscle after exercise. Another possible area for new research would be utilizing translational factors to measure MPD in humans.

Appendix A: Detailed Description of Research Methods and Procedures

Subject Selection and Screening

Forty males between the ages of 18-25 years were selected for this study. Subjects completed a health history questionnaire to screen for any contraindications to either strenuous resistance exercise or the biopsy and blood draw procedures. Since this study involved the use of milk, the questionnaire also screened subjects for food allergies, such as lactose intolerance. The only nutritional supplement allowed for subjects to consume was a multivitamin. Also, none of the subjects had participated in resistance exercise for a minimum of 3 months prior to this study. Finally, an informed consent form was read and signed by all the subjects.

Subject Pre-Testing

Subjects' 1RM for leg press and leg extension were determined at least 4 days prior to the acute. Subjects first completed 10 repetitions at a light weight to warm up and become accustomed to the equipment. This time was also used to instruct the subject on proper form and technique for the exercise. Subjects then performed one repetition of the exercise at progressively higher weights, with a rest period of 1-2 min between each attempt, until the subject could not complete the repetition without assistance. Four to five minutes were allowed for rest before testing the 1RM for leg extension. The subjects' dominant leg was tested for leg extension.

Urine Samples

All subjects collected total urine excreted in multiple polypropylene bottles containing 1 mL 50% hydrochloric acid as a preservative for 2 days. The collection days were the day before and the day of testing. Instructions were given to the subjects regarding proper collection and the completion of a meat-free diet during collection. Collections began with the second void of the day and were returned to the lab the following morning. The daily samples for each subject were mixed and total volume was

recorded. Then two 50-mL aliquots, for urinary 3MH and creatinine, were frozen at -20°C. Urine samples were thawed at room temperature and urinary 3MH was measured using the Waters Pico•Tag amino acid analyzer by the laboratory of Dr. Ken Webb in the Department of Animal and Poultry Science at Virginia Tech. Urinary creatinine was analyzed via spectrophotometery performing a manual assay with a commercially available kit (Sigma #555, Sigma Chemical Co., St. Louis, MO). All samples were analyzed in duplicate.

Muscle Samples

A certified clinical laboratory technician obtained two biopsies of the vastus lateralis from all subjects under local anesthesia using the percutaneous needle muscle biopsy technique with suction. A local anesthetic (lidocaine) was injected in several punctures following cleansing. After 4-5 min, a deep incision approximately 1 cm long was made in the leg. The needle was inserted into the incision and the sample was removed. To maximize sample size, a double pass was performed. The first biopsy was performed prior to exercise and the second an hour after the postexercise beverage was consumed. The biopsies were performed on the dominant leg, which was used for leg extension, approximately 2 cm from each other. The sample was immediately frozen in liquid nitrogen and stored in a freezer with a temperature of -80°.

After collecting all samples, they were mailed to Dr. Matthew Hickey at Colorado State University for analysis of muscle eIF. Using an anti-eIF4E monoclonal antibody, eIF4E was isolated by immunoprecipitation. The protein was electrophoretically transferred to a polyvinylidene difluoride membrane and incubated with polyclonal antipeptide antibody for eIF4E•eIF4G complex or eIF4E•4E-BP1 complex. The blots were then developed using Western blot technique (10).

Blood Collection and Analysis

A certified clinical laboratory technician obtained 15 mL blood samples from an arm vein of all subjects prior to exercise, immediately postexercise and 1-hr postexercise. All samples were placed in an ice bath for 30 min allowing the blood to clot. After centrifugation at 3,000 rpm for 15 min at 4°C, 2 mL of serum was placed in a 10cc high-speed centrifuge tube for amino acid analysis. The remaining serum was stored at -20°C until analysis. A 1:2 dilution was made with the serum and norleucine (NLE). The NLE was provided by the laboratory of Dr. Ken Webb with methanol added as an internal standard. These samples were stored for 24 hr with the remaining aliquots of serum at -20°C until analysis. After the 24-hr storage, the mixture of methanol and serum was centrifuged at 10,500 rpm for 20 min at 4°C. The supernatant was then refrigerated for later analysis of amino acids. Serum amino acids were measured using the Waters Pico•Tag amino acid analyzer by the laboratory of Dr. Ken Webb. Serum glucose was analyzed spectrophotometrically using manual assay procedures from a commercially available kit (Kit #315). Serum insulin and growth hormone (GH) were analyzed by radioimmunoassay using commercially available kits (Diagnostic Products Corp., Los Angeles, CA) in the laboratory of Dr. Frank Gwazdauskas in the Department of Dairy Science at Virginia Tech.

Acute Exercise Bout

Subjects completed 5 sets of 8 repetitions for leg press at 80% 1RM with 2 min rest between sets. Then subjects completed 5 sets of 8 repetitions for leg extension at 80% 1RM only using their dominant leg. Again, subjects were given 2 min to rest between sets. If a subject could not complete 5 sets at 80% 1RM for leg extension, the weight was decreased slightly to the maximum weight that the subject could lift for 8 repetitions.

Postexercise Nutritional Supplementation

Subjects received either a non-caloric placebo beverage or an experimental beverage consisting of 5 kcal · kg $^{-1}$ within 5 min after their resistance exercise bout. PL was a flavored non-caloric beverage. CE was made up of 1.25 g · kg $^{-1}$ of carbohydrate. MILK 1's composition was 0.92 g · kg $^{-1}$ carbohydrate, 0.21 g · kg $^{-1}$ protein, and 0.06 g · kg $^{-1}$ fat. MILK 2 was composed of 0.875 g · kg $^{-1}$ carbohydrate and 0.375 g · kg $^{-1}$ protein. MILK 2 was fortified with dry milk to be similar to MILK 1 in volume. The beverages were consumed in less than 5 min.

Appendix B: Raw Data Tables

Table 1: Anthropometrical Data for Subjects

Group	Subject #	Age (Years)	Height (m)	Weight (kg)	BMI	Body Fat (%)
MILK 1	1	20	1.708	74.66	25.59	16.72
MILK 1	5	23	1.702	70.91	24.48	10.54
MILK 1	10	20	1.753	85.00	27.66	6.61
MILK 1	16	21	1.867	89.00	25.53	11.82
MILK 1	19	22	1.664	71.75	25.91	6.20
MILK 1	23	24	1.93	107.75	28.93	32.11
MILK 1	26	22	1.803	98.50	30.30	24.10
MILK 1	29	22	1.753	77.75	25.30	5.88
MILK 1	34	20	1.638	67.50	25.16	7.45
MILK 1	39	25	1.803	72.00	22.15	3.97
	Mean	**21.9**	**1.762**	**81.48**	**26.10 ***	**12.54**
	± SEM	**0.547**	**0.029**	**4.22**	**0.733**	**2.90**
MILK 2	2	23	1.778	74.55	23.58	13.91
MILK 2	7	22	1.727	69.00	23.13	7.14
MILK 2	9	21	1.88	78.64	22.25	10.29
MILK 2	20	23	1.816	85.50	25.93	9.32
MILK 2	21	20	1.829	93.00	27.80	16.34
MILK 2	28	21	1.765	55.50	17.82	9.61
MILK 2	30	23	1.689	70.50	24.71	16.68
MILK 2	38	18	1.765	72.50	23.27	11.09
MILK 2	41	23	1.842	77.00	22.69	7.67
MILK 2	42	24	1.854	80.45	23.40	15.28
	Mean	**21.8**	**1.795**	**75.66**	**23.459**	**11.73**
	± SEM	**0.573**	**0.019**	**3.20**	**0.821**	**1.10**
CE	4	19	1.905	81.82	22.55	8.06
CE	6	20	1.778	63.60	20.12	10.01
CE	11	21	1.651	64.55	23.68	5.25
CE	15	22	1.854	80.00	23.27	7.46
CE	18	20	1.816	69.50	21.07	7.86
CE	24	22	1.765	67.25	21.59	13.51
CE	27	21	1.753	81.00	26.36	27.12
CE	31	18	1.651	54.75	20.09	5.35
CE	35	18	1.803	72.50	22.30	7.44
CE	37	21	1.702	59.50	20.54	11.82
	Mean	**20.2**	**1.768**	**69.45**	**22.156 ***	**10.39**
	± SEM	**0.467**	**0.026**	**2.95**	**0.615**	**2.00**
Placebo	3	20	1.803	72.27	22.23	11.31
Placebo	8	19	1.702	60.45	20.87	7.85
Placebo	12	20	1.765	67.00	21.51	7.96
Placebo	14	20	1.88	76.82	21.73	13.38
Placebo	17	23	1.778	79.00	24.99	6.52
Placebo	22	22	1.715	72.50	24.65	7.66
Placebo	25	22	1.765	86.50	27.77	23.60
Placebo	33	20	1.892	121.50	33.94	21.92
Placebo	40	24	1.778	84.00	26.57	27.01
	Mean	**21.1**	**1.786**	**80.00**	**24.917**	**14.14**
	± SEM	**0.564**	**0.022**	**5.84**	**1.382**	**2.60**

*– Indicates significant difference between groups ($p<0.05$).

Table 2: Individual Subject Glucose Data

Group	Subject	Concentration (mg/dl)		
		Pre	**IPE**	**1-Hr Post**
MILK 1	1	92.22	95.56	143.33
MILK 1	5	86.67	97.78	85.56
MILK 1	10	78.89	70	42.22
MILK 1	16	103.33	103.33	73.33
MILK 1	19	105.56	76.67	78.89
MILK 1	23	91.11	77.78	106.67
MILK 1	26	123.33	132.22	108.89
MILK 1	29	84.44	101.11	61.11
MILK 1	39	77.78	72.22	72.22
	Mean	**93.703**	**91.852**	**85.802**
	± SEM	**6.653**	**4.899**	**9.983**
MILK 2	2	100	102.22	55.56
MILK 2	7	97.78	92.22	63.33
MILK 2	9	47.78	62.22	41.11
MILK 2	20	84.44	93.33	70
MILK 2	21	83.33	98.89	84.44
MILK 2	30	97.78	113.33	108.89
MILK 2	38	87.78	95.56	121.11
MILK 2	41	88.89	120	92.22
MILK 2	42	84.44	78.89	72.22
	Mean	**85.802**	**95.184**	**78.764**
	± SEM	**5.214**	**5.741**	**8.521**
CE	6	88.89	66.67	45.56
CE	11	78.89	51.11	47.78
CE	15	87.78	76.67	87.78
CE	18	81.11	83.33	76.67
CE	24	78.89	88.89	65.56
CE	27	117.78	92.22	92.22
CE	31	112.22	84.44	107.78
CE	35	103.33	93.33	85.56
CE	37	88.89	100	163.33
	Mean	**93.087**	**81.851**	**85.804**
	± SEM	**4.846**	**5.041**	**11.842**
Placebo	8	55.56	53.33	41.11
Placebo	12	66.67	75.56	67.78
Placebo	14	98.89	82.22	90
Placebo	17	111.11	97.78	97.78
Placebo	22	98.89	84.44	74.44
Placebo	25	94.44	101.11	86.67
Placebo	32	110	104.44	114.44
Placebo	33	106.67	95.56	97.78
Placebo	40	87.78	84.44	100
	Mean	**92.223**	**86.542**	**85.556**
	± SEM	**6.447**	**5.259**	**7.244**

Results are not significantly different ($p>0.05$).

Table 3: Individual Subject Insulin Data

Group	Subject	Concentration (µIU/mL)		
		Pre	**IPE**	**1-Hr Post**
MILK 1	1	31.63	25.35	167.50
MILK 1	5	17.50	18.53	81.99
MILK 1	10	27.78	17.43	136.53
MILK 1	16	12.16	13.16	66.03
MILK 1	19	11.27	13.68	64.15
MILK 1	23	12.45	17.75	56.75
MILK 1	26	22.36	19.75	63.88
MILK 1	29	11.31	12.99	16.93
MILK 1	39	6.76	6.42	12.58
	Mean	**17.024**	**16.118**	**74.038** [AB]
	± SEM	**2.822**	**1.776**	**16.794**
MILK 2	2	15.68	25.91	43.38
MILK 2	7	11.15	10.95	32.61
MILK 2	9	11.51	11.92	70.32
MILK 2	20	10.42	11.29	30.90
MILK 2	21	11.44	11.25	58.60
MILK 2	30	11.44	14.36	59.39
MILK 2	38	19.29	25.71	81.71
MILK 2	41	12.58	9.62	35.46
MILK 2	42	14.97	16.74	49.09
	Mean	**13.164**	**15.306**	**51.273** [AB]
	± SEM	**0.970**	**2.105**	**5.880**
CE	4	17.34	15.88	105.63
CE	6	13.25	13.96	34.73
CE	11	10.56	10.01	17.66
CE	15	12.80	14.55	85.89
CE	18	10.55	10.48	42.94
CE	24	21.24	11.37	40.49
CE	27	23.82	31.20	28.33
CE	35	21.90	15.72	47.37
CE	37	15.88	11.14	108.47
	Mean	**16.371**	**14.923**	**56.834** [AB]
	± SEM	**1.671**	**2.168**	**11.351**
Placebo	8	14.81	12.38	11.57
Placebo	12	13.39	16.43	16.75
Placebo	14	27.71	27.28	18.47
Placebo	17	15.05	13.77	23.14
Placebo	22	10.53	9.43	10.82
Placebo	25	10.81	11.09	11.23
Placebo	32	23.11	15.08	14.75
Placebo	33	29.09	21.45	24.57
Placebo	40	13.99	15.49	18.67
	Mean	**17.610**	**15.822**	**16.663**
	± SEM	**2.374**	**1.838**	**1.687**

[A] – Indicates significant difference from Pre and IPE within group ($p<0.01$).
[B] – Indicates significant difference from Placebo 1-Hr Post ($p<0.05$).

Table 4: Individual Subject HGH Data

Group	Subject	Concentration (ηg/mL)		
		Pre	**IPE ***	**1-Hr Post**
MILK 1	5	1.00	2.50	1.00
MILK 1	10	1.13	3.44	1.11
MILK 1	16	1.00	2.74	1.22
MILK 1	19	1.00	2.94	1.00
MILK 1	23	1.00	1.00	1.00
MILK 1	26	1.00	1.90	1.00
MILK 1	29	1.00	6.07	1.00
MILK 1	39	1.18	2.85	1.00
	Mean	**1.038**	**2.931**	**1.042**
	± SEM	**0.025**	**0.520**	**0.029**
MILK 2	2	1.00	1.00	1.08
MILK 2	7	1.00	6.54	1.00
MILK 2	9	1.00	4.48	1.00
MILK 2	20	1.00	2.52	1.00
MILK 2	21	1.00	9.30	1.01
MILK 2	38	1.00	1.00	1.00
MILK 2	41	1.00	2.90	1.00
MILK 2	42	1.00	4.09	1.00
	Mean	**1.000**	**3.976**	**1.012**
	± SEM	**0.000**	**1.001**	**0.010**
CE	6	3.00	8.63	1.06
CE	11	1.02	1.85	1.00
CE	15	1.00	8.99	1.10
CE	18	1.00	1.95	1.00
CE	27	1.00	7.10	1.00
CE	31	1.00	11.42	1.00
CE	35	1.00	6.03	1.00
CE	37	1.06	1.05	1.00
	Mean	**1.260**	**5.876**	**1.021**
	± SEM	**0.249**	**1.366**	**0.014**
Placebo	8	1.00	5.20	1.00
Placebo	12	1.00	1.95	1.00
Placebo	17	1.28	2.58	1.00
Placebo	22	1.00	4.73	1.00
Placebo	25	1.00	2.67	1.00
Placebo	32	1.00	1.00	1.00
Placebo	33	1.00	3.05	1.01
Placebo	40	1.00	6.49	1.01
	Mean	**1.030**	**3.460**	**1.004**
	± SEM	**0.035**	**0.650**	**0.002**

* – Indicates significant difference between this time point and all others ($p<0.05$).

Table 5: Individual Subject 3MH/Creatinine Data

Group	Subject	3MH/Creatinine (µmol/g)	
		Day 1	**Day 2**
MILK 1	1	273.334	91.396
MILK 1	5	116.303	70.023
MILK 1	16	476.374	160.643
MILK 1	19	81.643	42.201
MILK 1	23	367.728	145.173
MILK 1	26	19.883	21.079
MILK 1	29	139.964	131.030
	Mean	**210.747**	**94.507 ***
	± SEM	**56.656**	**58.387**
MILK 2	2	76.164	30.904
MILK 2	7	508.639	141.622
MILK 2	20	204.199	65.500
MILK 2	28	36.494	21.194
MILK 2	38	221.192	64.750
MILK 2	41	233.533	74.530
MILK 2	42	31.639	32.363
	Mean	**187.409**	**61.552 ***
	± SEM	**101.503**	**19.710**
CE	4	63.305	38.626
CE	6	66.683	29.033
CE	11	508.451	260.979
CE	15	117.811	42.642
CE	27	22.275	20.647
CE	35	399.241	160.733
CE	37	34.442	29.017
	Mean	**173.173**	**83.097 ***
	± SEM	**90.835**	**32.677**
Placebo	12	396.970	129.229
Placebo	14	26.428	26.199
Placebo	22	217.693	131.065
Placebo	25	237.087	123.549
Placebo	32	326.595	128.432
Placebo	33	714.249	269.979
Placebo	40	39.229	34.693
	Mean	**279.750**	**120.449 ***
	± SEM	**143.590**	**39.754**

* – Indicates significant difference from Day 1 ($p<0.001$).

Table 6: Individual Subject 24-Hr Urine Volume Data

Group	Subject	24-Hr Urine Volume (L)	
		Day 1	**Day 2**
MILK 1	1	0.510	1.850
MILK 1	5	0.950	1.000
MILK 1	16	0.980	1.280
MILK 1	19	0.830	1.740
MILK 1	23	2.350	2.520
MILK 1	26	1.320	1.950
MILK 1	29	1.720	1.060
MILK 1	34	1.200	1.850
	Mean	**1.233**	**1.656**
	± SEM	**0.204**	**0.181**
MILK 2	2	1.550	1.560
MILK 2	7	0.900	1.210
MILK 2	9	1.690	1.590
MILK 2	20	1.440	1.340
MILK 2	21	0.950	1.760
MILK 2	28	1.400	1.520
MILK 2	30	0.780	0.650
MILK 2	38	0.300	0.310
MILK 2	41	0.890	2.300
MILK 2	42	0.770	0.900
	Mean	**1.067**	**1.314**
	± SEM	**0.138**	**0.182**
CE	4	1.140	0.710
CE	6	0.830	1.000
CE	11	1.050	2.700
CE	15	0.370	0.800
CE	18	0.810	2.500
CE	27	0.870	0.460
CE	31	0.880	0.850
CE	35	1.790	1.620
CE	37	1.230	0.975
	Mean	**0.997**	**1.291**
	± SEM	**0.129**	**0.269**
Placebo	12	1.700	1.580
Placebo	14	0.850	0.520
Placebo	17	2.880	2.820
Placebo	22	0.720	1.380
Placebo	25	1.600	1.200
Placebo	32	0.710	1.460
Placebo	33	0.940	1.160
Placebo	40	1.860	1.020
	Mean	**1.408**	**1.393**
	± SEM	**0.267**	**0.234**

Results are not significantly different ($p>0.05$).

Table 7: Individual Subject Creatinine Data

Group	Subject	Creatinine (g)	
		Day 1	**Day 2**
MILK 1	1	1.194	1.937
MILK 1	5	1.811	1.798
MILK 1	16	1.922	1.930
MILK 1	19	1.328	1.957
MILK 1	23	1.883	1.705
MILK 1	26	2.052	2.007
MILK 1	29	1.777	1.628
MILK 1	34	1.573	0.886
	Mean	**1.693**	**1.731**
	± SEM	**0.106**	**0.130**
MILK 2	2	1.528	1.730
MILK 2	7	1.148	1.436
MILK 2	9	1.495	1.389
MILK 2	20	1.772	1.514
MILK 2	21	2.479	1.786
MILK 2	28	1.156	0.869
MILK 2	30	1.163	1.592
MILK 2	38	0.792	0.519
MILK 2	41	1.917	1.911
MILK 2	42	0.912	0.832
	Mean	**1.436**	**1.358**
	± SEM	**0.162**	**0.147**
CE	4	1.390	1.859
CE	6	1.139	1.346
CE	11	1.640	1.527
CE	15	1.155	0.927
CE	18	1.417	1.615
CE	27	1.073	0.594
CE	31	1.861	0.722
CE	35	1.662	1.971
CE	37	1.140	0.862
	Mean	**1.386**	**1.269**
	± SEM	**0.094**	**0.170**
Placebo	12	1.202	1.425
Placebo	14	0.597	0.514
Placebo	17	1.700	1.761
Placebo	22	1.493	2.118
Placebo	25	2.396	1.817
Placebo	32	1.433	1.725
Placebo	33	1.666	1.595
Placebo	40	1.339	1.533
	Mean	**1.478**	**1.561**
	± SEM	**0.179**	**0.167**

Results are not significantly different ($p>0.05$).

Table 8: Individual Subject Total AA Data

Group	Subject	Concentration (mg/L)	
		Pre	**1-Hr Post**
MILK 1	1	435.705	446.740
MILK 1	5	372.160	414.060
MILK 1	10	433.290	462.555
MILK 1	16	407.375	449.660
MILK 1	19	415.580	406.760
MILK 1	23	424.280	445.700
MILK 1	26	445.790	481.760
MILK 1	29	382.160	472.185
MILK 1	34	520.485	596.555
MILK 1	39	391.730	465.515
	Mean	**422.856**	**464.149** [ABC]
	± SEM	**13.259**	**16.521**
MILK 2	2	377.085	486.155
MILK 2	7	382.430	486.320
MILK 2	9	425.515	450.580
MILK 2	20	381.075	447.045
MILK 2	21	390.805	454.855
MILK 2	28	423.200	466.780
MILK 2	30	383.300	438.185
MILK 2	38	405.305	525.445
MILK 2	41	414.825	454.780
MILK 2	42	463.095	486.025
	Mean	**404.664**	**469.617** [ABC]
	± SEM	**8.664**	**8.330**
CE	4	370.395	405.320
CE	6	354.400	360.925
CE	11	412.900	319.745
CE	15	391.090	329.935
CE	18	334.315	290.750
CE	24	409.595	330.090
CE	27	390.970	331.980
CE	31	369.930	467.845
CE	35	407.550	364.780
CE	37	427.840	377.850
	Mean	**386.899**	**357.922** [A]
	± SEM	**9.255**	**15.980**
Placebo	8	393.365	379.350
Placebo	12	386.185	357.665
Placebo	14	423.665	375.105
Placebo	17	358.700	322.035
Placebo	22	361.475	374.250
Placebo	25	374.895	370.165
Placebo	32	435.035	432.080
Placebo	33	461.040	491.915
Placebo	40	437.250	396.035
	Mean	**403.512**	**388.733**
	± SEM	**12.273**	**16.168**

[A] – Indicates significant difference from Pre ($p<0.05$).
[B] – Indicates significant difference from Placebo 1-Hr Post ($p<0.001$).
[C] – Indicates significant difference from CE 1-Hr Post ($p<0.001$).

Table 9: Individual Subject Essential AA Data

Group	Subject	Concentration (mg/L)	
		Pre	**1-Hr Post**
MILK 1	1	178.985	169.970
MILK 1	5	148.425	157.110
MILK 1	10	192.305	207.430
MILK 1	16	160.500	173.910
MILK 1	19	167.805	157.010
MILK 1	23	166.780	182.190
MILK 1	26	164.645	175.065
MILK 1	29	150.490	182.735
MILK 1	34	197.130	218.755
MILK 1	39	139.800	182.790
	Mean	**166.687**	**180.697** [ABC]
	± SEM	**5.866**	**6.222**
MILK 2	2	155.185	175.585
MILK 2	7	159.040	183.375
MILK 2	9	147.380	154.115
MILK 2	20	167.560	194.330
MILK 2	21	157.245	180.495
MILK 2	28	150.865	171.175
MILK 2	30	137.795	155.600
MILK 2	38	139.480	206.190
MILK 2	41	139.355	144.560
MILK 2	42	175.195	182.115
	Mean	**152.910**	**174.754** [ABC]
	± SEM	**3.944**	**6.010**
CE	4	153.295	130.150
CE	6	130.605	124.610
CE	11	164.295	112.495
CE	15	149.360	112.390
CE	18	134.755	105.085
CE	24	148.410	104.810
CE	27	141.595	104.740
CE	31	102.670	150.055
CE	35	142.510	117.205
CE	37	157.350	123.235
	Mean	**142.485**	**118.480** [AB]
	± SEM	**5.460**	**4.501**
Placebo	8	150.685	138.470
Placebo	12	154.075	130.395
Placebo	14	163.740	136.505
Placebo	17	149.715	124.725
Placebo	22	141.395	138.960
Placebo	25	140.320	129.700
Placebo	32	144.635	132.300
Placebo	33	172.400	182.550
Placebo	40	163.910	136.130
	Mean	**153.431**	**138.859** [AC]
	± SEM	**3.711**	**5.677**

[A] – Indicates significant difference from Pre ($p<0.05$).
[B] – Indicates significant difference from Placebo 1-Hr Post ($p<0.001$).
[C] – Indicates significant difference from CE 1-Hr Post ($p<0.001$).

Table 10: Individual Subject BCAA Data

Group	Subject	Concentration (mg/L)	
		Pre	**1-Hr Post**
MILK 1	1	78.245	70.675
MILK 1	5	62.920	64.245
MILK 1	10	77.600	88.530
MILK 1	16	56.870	60.610
MILK 1	19	61.895	59.160
MILK 1	23	67.635	70.900
MILK 1	26	56.855	60.370
MILK 1	29	50.020	63.615
MILK 1	34	78.775	89.540
MILK 1	39	44.645	63.415
	Mean	**63.546**	**69.106** [BC]
	± SEM	**3.793**	**3.550**
MILK 2	2	66.420	64.745
MILK 2	7	63.400	70.900
MILK 2	9	55.465	58.795
MILK 2	20	62.505	69.380
MILK 2	21	57.360	65.855
MILK 2	28	54.605	64.240
MILK 2	30	55.125	63.425
MILK 2	38	59.765	93.595
MILK 2	41	53.565	54.140
MILK 2	42	58.875	60.585
	Mean	**58.709**	**66.566** [A,BC]
	± SEM	**1.356**	**3.378**
CE	4	57.120	46.235
CE	6	48.415	40.825
CE	11	64.355	34.450
CE	15	51.590	34.430
CE	18	50.460	32.795
CE	24	61.590	38.700
CE	27	56.175	36.565
CE	31	37.625	59.805
CE	35	53.165	35.775
CE	37	56.255	37.095
	Mean	**53.675**	**39.668** [A]
	± SEM	**2.361**	**2.548**
Placebo	8	56.720	46.245
Placebo	12	59.175	45.710
Placebo	14	67.305	52.040
Placebo	17	64.725	51.245
Placebo	22	52.245	49.755
Placebo	25	50.855	39.370
Placebo	32	59.650	46.110
Placebo	33	70.270	68.375
Placebo	40	57.650	42.980
	Mean	**59.844**	**49.092** [A]
	± SEM	**2.179**	**2.754**

[A] – Indicates significant difference from Pre ($p<0.05$).
[B] – Indicates significant difference from Placebo 1-Hr Post ($p<0.001$).
[C] – Indicates significant difference from CE 1-Hr Post ($p<0.001$).

Table 11: Individual Subject Leucine Data

Group	Subject	Concentration (mg/L)	
		Pre	**1-Hr Post**
MILK 1	1	26.315	13.225
MILK 1	5	19.970	21.230
MILK 1	10	24.235	30.525
MILK 1	16	20.570	22.035
MILK 1	19	21.960	20.850
MILK 1	23	22.915	24.365
MILK 1	26	18.800	20.350
MILK 1	29	18.195	23.315
MILK 1	34	26.845	32.420
MILK 1	39	16.175	23.720
	Mean	**21.598**	**23.204** [BC]
	± SEM	**1.111**	**1.695**
MILK 2	2	22.335	21.500
MILK 2	7	22.515	24.975
MILK 2	9	18.720	20.475
MILK 2	20	22.525	25.120
MILK 2	21	20.055	23.730
MILK 2	28	18.865	23.030
MILK 2	30	19.285	22.655
MILK 2	38	19.930	33.335
MILK 2	41	18.170	18.035
MILK 2	42	21.610	21.930
	Mean	**20.401**	**23.479** [ABC]
	± SEM	**0.537**	**1.282**
CE	4	20.165	16.100
CE	6	16.105	12.995
CE	11	22.295	10.465
CE	15	18.585	11.295
CE	18	17.595	10.475
CE	24	20.645	11.645
CE	27	19.160	11.390
CE	31	10.805	19.625
CE	35	17.545	10.545
CE	37	19.320	11.820
	Mean	**18.222**	**12.635** [A]
	± SEM	**0.993**	**0.941**
Placebo	8	19.490	15.350
Placebo	12	19.605	14.435
Placebo	14	22.320	16.485
Placebo	17	20.775	15.960
Placebo	22	17.655	16.560
Placebo	25	17.635	12.870
Placebo	32	19.660	13.790
Placebo	33	23.040	21.630
Placebo	40	19.490	13.670
	Mean	**19.963**	**15.639** [A]
	± SEM	**0.613**	**0.866**

[A] – Indicates significant difference from Pre ($p<0.05$).
[B] – Indicates significant difference from Placebo 1-Hr Post ($p<0.001$).
[C] – Indicates significant difference from CE 1-Hr Post ($p<0.001$).

Table 12: Individual Subject Isoleucine Data

Group	Subject	Concentration (mg/L)	
		Pre	**1-Hr Post**
MILK 1	1	12.850	19.965
MILK 1	5	9.940	10.450
MILK 1	10	13.095	15.365
MILK 1	16	9.935	10.735
MILK 1	19	11.260	10.560
MILK 1	23	13.230	13.290
MILK 1	26	10.690	11.500
MILK 1	29	8.010	10.745
MILK 1	34	14.910	16.150
MILK 1	39	7.980	11.990
	Mean	**11.190**	**13.075** [ABC]
	± SEM	**0.732**	**1.000**
MILK 2	2	10.850	10.610
MILK 2	7	10.230	12.025
MILK 2	9	9.920	10.695
MILK 2	20	10.615	12.160
MILK 2	21	9.925	11.335
MILK 2	28	10.055	12.545
MILK 2	30	9.865	11.540
MILK 2	38	11.060	18.525
MILK 2	41	9.830	10.485
MILK 2	42	10.180	10.815
	Mean	**10.253**	**12.074** [ABC]
	± SEM	**0.139**	**0.752**
CE	4	8.985	7.460
CE	6	8.705	6.880
CE	11	10.255	4.355
CE	15	8.265	4.450
CE	18	8.485	4.775
CE	24	10.580	5.605
CE	27	11.545	6.555
CE	31	5.845	10.590
CE	35	8.955	5.345
CE	37	9.940	5.615
	Mean	**9.156**	**6.163** [A]
	± SEM	**0.495**	**0.590**
Placebo	8	9.300	7.435
Placebo	12	10.020	7.065
Placebo	14	12.625	9.430
Placebo	17	10.350	7.700
Placebo	22	9.645	8.560
Placebo	25	8.570	5.775
Placebo	32	10.935	7.515
Placebo	33	13.350	12.245
Placebo	40	9.830	6.685
	Mean	**10.514**	**8.046** [A]
	± SEM	**0.519**	**0.630**

[A] – Indicates significant difference from Pre ($p<0.05$).
[B] – Indicates significant difference from Placebo 1-Hr Post ($p<0.001$).
[C] – Indicates significant difference from CE 1-Hr Post ($p<0.001$).

Table 13: Individual Subject Valine Data

Group	Subject	Concentration (mg/L)	
		Pre	**1-Hr Post**
MILK 1	1	39.080	37.485
MILK 1	5	33.010	32.565
MILK 1	10	40.270	42.640
MILK 1	16	26.365	27.840
MILK 1	19	28.675	27.750
MILK 1	23	31.490	33.245
MILK 1	26	27.365	28.520
MILK 1	29	23.815	29.555
MILK 1	34	37.020	40.970
MILK 1	39	20.490	27.705
	Mean	**30.758**	**32.828** [BC]
	± SEM	**2.091**	**1.797**
MILK 2	2	33.235	32.635
MILK 2	7	30.655	33.900
MILK 2	9	26.825	27.625
MILK 2	20	29.365	32.100
MILK 2	21	27.380	30.790
MILK 2	28	25.685	28.665
MILK 2	30	25.975	29.230
MILK 2	38	28.775	41.735
MILK 2	41	25.565	25.620
MILK 2	42	27.085	27.840
	Mean	**28.055**	**31.014** [ABC]
	± SEM	**0.781**	**1.439**
CE	4	27.970	22.675
CE	6	23.605	20.950
CE	11	31.805	19.630
CE	15	24.740	18.685
CE	18	24.380	17.545
CE	24	30.365	21.450
CE	27	25.470	18.620
CE	31	20.975	29.590
CE	35	26.665	19.885
CE	37	26.995	19.660
	Mean	**26.297**	**20.869** [A]
	± SEM	**1.015**	**1.078**
Placebo	8	27.930	23.460
Placebo	12	29.550	24.210
Placebo	14	32.360	26.125
Placebo	17	33.600	27.585
Placebo	22	24.945	24.635
Placebo	25	24.650	20.725
Placebo	32	29.055	24.805
Placebo	33	33.880	34.500
Placebo	40	28.330	22.625
	Mean	**29.367**	**25.408** [A]
	± SEM	**1.132**	**1.311**

[A] – Indicates significant difference from Pre ($p<0.05$).
[B] – Indicates significant difference from Placebo 1-Hr Post ($p<0.001$).
[C] – Indicates significant difference from CE 1-Hr Post ($p<0.05$).

Table 14: Summary of Mean Concentration of Amino Acids (mg/L)

	Beverage Groups							
	Milk 1		Milk 2		CE		PL	
Amino Acid	**Pre**	**1-Hr Post**	**Pre**	**1-Hr Post**	**Pre**	**1-Hr Post**	**Pre**	**1-Hr Post**
Alanine	36.675	51.052	36.581	56.320	34.917	39.575	39.207	42.284
Arginine	16.633	17.191	16.006	17.371	15.559	14.122	17.070	16.341
Asparagine	5.897	7.673	5.757	8.320	5.038	5.181	5.080	5.577
Aspartate	2.593	2.360	2.453	2.265	2.780	2.215	2.342	1.741
Cysteine	0.515	1.391	0.487	0.859	0.893	0.959	0.495	0.740
Glutamate	3.923	4.996	3.828	4.100	3.163	3.792	4.519	4.410
Glutamine	93.741	92.429	90.978	96.409	93.487	87.697	90.061	91.649
Glycine	19.682	19.251	18.538	19.307	17.442	17.156	17.930	18.119
Histidine	14.098	15.301	12.623	13.177	12.295	11.814	11.831	12.772
Isoleucine	11.338	13.682	10.249	12.155	9.222	7.159	10.272	8.355
Leucine	21.681	23.644	20.179	23.242	17.678	13.975	19.495	16.195
Lysine	21.554	26.021	18.936	24.128	17.984	17.002	20.079	20.106
Methionine	5.216	5.846	4.538	5.756	4.406	3.757	5.087	4.529
Phenylalanine	11.081	12.129	10.268	10.448	9.641	8.195	11.814	14.122
Proline	29.928	38.464	32.714	43.474	30.092	30.434	28.491	29.091
Serine	9.569	10.456	9.614	11.385	9.615	8.716	8.649	8.201
Threonine	17.229	18.677	15.788	18.559	14.196	14.079	15.434	16.378
Tryptophan	16.921	17.929	13.974	15.848	12.505	11.830	13.524	13.325
Tyrosine	15.362	17.605	13.287	15.575	13.333	11.046	14.192	13.185
Valine	31.568	34.086	27.975	30.906	26.006	22.355	28.132	25.555

Table 15: Individual Subject eIF4E•eIF4G Data

Group	Subject	Concentration	
		Pre	**1-Hr Post**
MILK 1	1	241.56	240.49
MILK 1	5	142.02	138.95
MILK 1	10	153.03	158.42
MILK 1	16	212.04	211.82
MILK 1	19	200.43	206.93
MILK 1	23	179.70	198.33
MILK 1	26	201.56	204.68
MILK 1	29	192.94	198.60
MILK 1	34	72.20	70.11
MILK 1	39	171.34	198.37
	Mean	**176.68**	**182.67**
	± SEM	**14.77**	**15.30**
MILK 2	2	235.66	242.29
MILK 2	7	250.27	240.91
MILK 2	9	149.24	164.17
MILK 2	20	162.43	203.52
MILK 2	21	201.28	200.15
MILK 2	28	105.36	184.75
MILK 2	30	166.65	172.35
MILK 2	38	244.12	247.81
MILK 2	41	173.93	158.82
MILK 2	42	242.36	243.25
	Mean	**193.13**	**205.80**
	± SEM	**15.57**	**11.19**
CE	4	144.36	179.02
CE	6	218.03	217.34
CE	11	233.79	239.53
CE	15	236.56	233.88
CE	18	143.12	182.59
CE	24	155.56	125.69
CE	27	70.33	76.92
CE	31	209.44	209.58
CE	35	154.82	120.94
CE	37	146.26	178.94
	Mean	**171.23**	**176.44**
	± SEM	**16.55**	**16.89**
Placebo	3	162.56	170.43
Placebo	8	211.75	212.08
Placebo	12	239.32	234.64
Placebo	14	155.99	191.07
Placebo	17	180.13	177.74
Placebo	22	190.62	199.61
Placebo	25	145.13	193.37
Placebo	32	182.83	165.40
Placebo	33	174.74	184.52
Placebo	40	171.97	171.37
	Mean	**181.50**	**190.02**
	± SEM	**8.70**	**6.75**

Results are significantly not different ($p>0.05$).

Table 16: Individual Subject eIF4E•4E-BP1 Data

Group	Subject	Concentration	
		Pre	**1-Hr Post**
MILK 1	1	247.40	225.55
MILK 1	5	133.26	128.59
MILK 1	10	154.38	162.30
MILK 1	16	184.26	187.31
MILK 1	19	190.32	179.11
MILK 1	23	163.28	163.07
MILK 1	26	174.88	182.73
MILK 1	29	158.66	148.51
MILK 1	34	88.93	95.33
MILK 1	39	143.14	138.34
	Mean	**163.85**	**161.08**
	± SEM	**13.04**	**11.40**
MILK 2	2	251.54	247.79
MILK 2	7	223.30	219.62
MILK 2	9	153.89	145.13
MILK 2	20	178.76	163.75
MILK 2	21	174.13	153.48
MILK 2	28	112.30	175.07
MILK 2	30	146.71	148.25
MILK 2	38	245.52	243.22
MILK 2	41	156.40	146.17
MILK 2	42	239.58	239.86
	Mean	**188.21**	**188.23**
	± SEM	**15.32**	**13.92**
CE	4	129.21	152.93
CE	6	214.08	207.38
CE	11	252.20	251.57
CE	15	238.12	241.97
CE	18	204.35	201.79
CE	24	198.90	204.59
CE	27	35.75	51.13
CE	31	168.81	151.11
CE	35	181.38	71.13
CE	37	143.13	144.46
	Mean	**176.59**	**167.81**
	± SEM	**19.85**	**21.24**
Placebo	3	189.73	182.58
Placebo	8	117.69	110.96
Placebo	12	212.79	221.17
Placebo	14	149.27	140.73
Placebo	17	184.66	168.66
Placebo	22	156.95	160.28
Placebo	25	141.06	175.69
Placebo	32	144.57	147.63
Placebo	33	153.87	131.43
Placebo	40	158.87	161.59
	Mean	**160.95**	**160.07**
	± SEM	**8.71**	**9.62**

Results are not significantly different ($p>0.05$).

Table 17: Individual Subject Likert Data – Taste

Group	Subject	Score	
		IPE	**1-Hr Post**
MILK 1	1	13.5	13.25
MILK 1	5	8.5	8.5
MILK 1	10	12.5	11.75
MILK 1	16	13.25	13
MILK 1	19	12.25	11.5
MILK 1	23	13.5	13.25
MILK 1	26	10.5	11
MILK 1	29	12.25	10.5
MILK 1	34	9	7.75
MILK 1	39	12.5	10.5
	Mean	**11.78** [A]	**11.10** [A]
	± SEM	**0.57**	**0.60**
MILK 2	2	12.5	12
MILK 2	7	12.75	12.25
MILK 2	9	13.5	13
MILK 2	20	15	15
MILK 2	21	13	11.25
MILK 2	28	12.25	11.5
MILK 2	30	8.5	7
MILK 2	38	11	11.5
MILK 2	41	8.5	10.25
MILK 2	42	12	11.5
	Mean	**11.90** [A]	**11.53** [A]
	± SEM	**0.65**	**0.64**
CE	4	0.5	0.75
CE	6	13.5	13
CE	11	10	10
CE	15	6	9.75
CE	18	2	2
CE	24	13.25	12.5
CE	27	13	13
CE	31	10.25	10.25
CE	35	10.5	10.5
CE	37	13	10.25
	Mean	**9.20**	**9.20**
	± SEM	**1.51**	**1.37**
Placebo	3	7.5	5.5
Placebo	8	9	9.5
Placebo	12	10	9
Placebo	14	1	0.75
Placebo	17	9.5	10
Placebo	22	12.5	12
Placebo	25	11	11.25
Placebo	32	9	9.25
Placebo	33	8	8.5
Placebo	40	7	10
	Mean	**8.45**	**8.58**
	± SEM	**0.98**	**1.03**

[A] – Indicates significant difference from CE and Placebo ($p<0.05$)

Table 18: Individual Subject Likert Data – Quenches Thirst

Group	Subject	Score	
		IPE	**1-Hr Post**
MILK 1	1	13.75	13.25
MILK 1	5	3.5	4
MILK 1	10	3.5	6.5
MILK 1	16	11.25	11
MILK 1	19	8.5	6
MILK 1	23	7.5	5.5
MILK 1	26	6.25	4
MILK 1	29	8.5	9.5
MILK 1	34	6.5	9.25
MILK 1	39	8.5	9
	Mean	**7.78**	**7.80**
	± SEM	**1.00**	**0.97**
MILK 2	2	9.75	10.5
MILK 2	7	8.5	8
MILK 2	9	10.25	11.75
MILK 2	20	13.5	15
MILK 2	21	9.5	12
MILK 2	28	11	11.5
MILK 2	30	9	7
MILK 2	38	11	10.25
MILK 2	41	10	10.25
MILK 2	42	10.25	10.5
	Mean	**10.28**	**10.68**
	± SEM	**0.44**	**0.69**
CE	4	0.5	1
CE	6	10.5	11
CE	11	14	12.75
CE	15	11.5	11.25
CE	18	3.5	7
CE	24	10.75	10.5
CE	27	13.5	13
CE	31	13.5	13.75
CE	35	10.25	10.25
CE	37	1	10.5
	Mean	**8.90**	**10.10**
	± SEM	**1.65**	**1.17**
Placebo	3	4	5.5
Placebo	8	10.5	8
Placebo	12	9	8.25
Placebo	14	14	13.5
Placebo	17	9.75	8.25
Placebo	22	13	12.5
Placebo	25	12.75	11
Placebo	32	11	11.5
Placebo	33	10.5	11
Placebo	40	13.5	13
	Mean	**10.80**	**10.25**
	± SEM	**0.92**	**0.83**

Results are not significantly different ($p>0.05$).

Table 19: Individual Subject Likert Data – Fulfills Hunger

Group	Subject	Score	
		IPE	**1-Hr Post**
MILK 1	1	13.75	6.5
MILK 1	5	8	10.75
MILK 1	10	2.5	6.5
MILK 1	16	3.5	7.25
MILK 1	19	9.75	7.25
MILK 1	23	10.25	10.25
MILK 1	26	10.5	13.25
MILK 1	29	8.25	7.75
MILK 1	34	9.5	10.25
MILK 1	39	8.75	6
	Mean	**8.48**	**8.58**
	± SEM	**1.05**	**0.76**
MILK 2	2	8.75	10.5
MILK 2	7	9.25	9.25
MILK 2	9	9.5	12
MILK 2	20	15	14.5
MILK 2	21	10.25	12.5
MILK 2	28	7.25	9.5
MILK 2	30	9.25	7.25
MILK 2	38	8.75	7.75
MILK 2	41	10.5	10
MILK 2	42	10	10.25
	Mean	**9.85**	**10.35**
	± SEM	**0.64**	**0.69**
CE	4	0.5	2
CE	6	10.25	11
CE	11	0.5	4
CE	15	9.5	11.25
CE	18	3	7
CE	24	2	2.75
CE	27	8.5	6.75
CE	31	10.25	13.5
CE	35	8.5	8.25
CE	37	0.75	10.5
	Mean	**5.38**	**7.70**
	± SEM	**1.37**	**1.24**
Placebo	3	5.5	4.5
Placebo	8	3.25	4
Placebo	12	10	9
Placebo	14	1.25	13.75
Placebo	17	6	6
Placebo	22	12.5	12.5
Placebo	25	7.5	7
Placebo	32	6.75	7.5
Placebo	33	4	2.5
Placebo	40	10.5	10.25
	Mean	**6.73**	**7.70**
	± SEM	**1.11**	**1.17**

Results are not significantly different ($p>0.05$).

Table 20: Individual Subject Likert Data – Energizing

Group	Subject	Score	
		IPE	**1-Hr Post**
MILK 1	1	4.5	9.5
MILK 1	5	6.5	8.25
MILK 1	10	1	4
MILK 1	16	1.5	8.5
MILK 1	19	4	4.75
MILK 1	23	8.5	7.25
MILK 1	26	12.25	12.25
MILK 1	29	7.5	7
MILK 1	34	7	7.25
MILK 1	39	9	6.25
	Mean	**6.18** [A]	**7.50** [A]
	± SEM	**1.10**	**0.74**
MILK 2	2	6	7.5
MILK 2	7	8	8.25
MILK 2	9	9.75	11
MILK 2	20	13.5	12
MILK 2	21	11.5	12.5
MILK 2	28	11	12
MILK 2	30	6.5	7.25
MILK 2	38	12.5	12
MILK 2	41	8.25	9.5
MILK 2	42	9.75	10.25
	Mean	**9.68**	**10.23**
	± SEM	**0.79**	**0.63**
CE	4	4.5	4
CE	6	10.5	11
CE	11	0.25	1.75
CE	15	10.25	11.75
CE	18	4.5	8
CE	24	10.5	10.5
CE	27	13.5	12
CE	31	13.75	6.75
CE	35	9.25	8.25
CE	37	0.75	13.25
	Mean	**7.78**	**8.73**
	± SEM	**1.56**	**1.18**
Placebo	3	5	4
Placebo	8	6.5	6.75
Placebo	12	8.25	8
Placebo	14	14	13.5
Placebo	17	7.5	6
Placebo	22	8	7.75
Placebo	25	8.5	8.25
Placebo	32	7.5	7
Placebo	33	3	4
Placebo	40	13.75	13.75
	Mean	**8.20**	**7.90**
	± SEM	**1.08**	**1.06**

[A] – Indicates significant difference MILK 2 ($p<0.05$).

Appendix C: Statistical Procedures and Results

The main effect of group, time, and the interaction of groups by time for urinary 3MH, serum glucose, amino acids, and hormones were determined by a two way ANOVA with repeated measures (Sigma Stat 2.03, SPSS Inc.). Tukey Post Hoc analysis was used when a significant F-ratio was calculated. Analysis of muscle eIF was performed in the laboratory of Dr. Matthew Hickey at Colorado State University. Significance was defined as $p<0.05$.

Table 21: ANOVA for Serum Glucose (mg/dL)

Source	DF	SS	MS	F	p
Beverage	3	213.8	71.2	0.07	0.975
Time	2	683.8	341.9	1.61	0.208
Beverage * Time	6	1643.4	273.9	1.29	0.274
Total	107	48248.8	450.9		

Table 22: ANOVA for Serum Insulin (µIU/mL)

Source	DF	SS	MS	F	p
Beverage	3	5684.5	1894.8	3.25	0.034
Time	2	26648.8	13324.4	42.94	<0.001
Beverage * Time	6	10667.7	1778.0	5.73	<0.001
Total	106	82117.4	774.7		

Table 23: ANOVA for Serum Growth Hormone (ηg/mL)

Source	DF	SS	MS	F	p
Beverage	3	13.7	4.6	1.75	0.177
Time	2	191.2	95.6	39.38	<0.001
Beverage * Time	6	23.5	3.9	1.61	0.158
Total	107	467.3	4.4		

Table 24: ANOVA for Creatinine Excretion (g/L)

Source	DF	SS	MS	F	p
Beverage	3	1.4	0.5	1.47	0.243
Day	1	0.003	0.003	0.04	0.834
Beverage * Day	3	0.1	0.02	0.31	0.816
Total	77	12.8	0.2		

Table 25: ANOVA for 3-Methylhistidine Excretion (µmol/L)

Source	DF	SS	MS	F	p
Beverage	3	102533.8	34177.9	0.69	0.568
Day	1	301873.6	301873.6	19.07	<0.001
Beverage * Day	3	29446.6	9815.5	0.62	0.607
Total	77	2411843.5	35997.7		

Table 26: ANOVA for 3-Methylhistidine-to-Creatinine ratio (µmol/g)

Source	DF	SS	MS	F	p
Beverage	3	36803.1	12267.7	0.42	0.740
Day	1	190030.7	190030.7	17.69	<0.001
Beverage * Day	3	8148.1	2716.0	0.25	0.859
Total	77	1637771.9	21269.8		

Table 27: ANOVA for Serum Total Amino Acids (mg/L)

Source	DF	SS	MS	F	p
Beverage	3	68333.8	22777.9	9.31	<0.001
Time	1	4749.6	4749.6	5.92	0.020
Beverage * Time	3	29555.3	9851.8	12.28	<0.001
Total	77	216881.9	2816.6		

Table 28: ANOVA for Serum Essential Amino Acids (mg/L)

Source	DF	SS	MS	F	p
Beverage	3	21856.1	7285.4	20.79	<0.001
Time	1	9.0	9.0	0.05	0.830
Beverage * Time	3	7202.3	2400.7	12.38	<0.001
Total	77	48110.3	624.8		

Table 29: ANOVA for Serum Branched-Chain Amino Acids (mg/L)

Source	DF	SS	MS	F	p
Beverage	3	4595.7	1531.9	13.83	<0.001
Time	1	156.4	156.4	3.18	0.083
Beverage * Time	3	1829.4	609.8	12.41	<0.001
Total	77	12155.2	157.9		

Table 30: ANOVA for Serum Leucine (mg/L)

Source	DF	SS	MS	F	p
Beverage	3	670.4	223.5	19.00	<0.001
Time	1	33.2	33.2	3.05	0.020
Beverage * Time	3	271.0	90.3	8.30	<0.001
Total	77	1764.3	22.9		

Table 31: ANOVA for Serum Isoleucine (mg/L)

Source	DF	SS	MS	F	p
Beverage	3	236.4	78.8	13.57	<0.001
Time	1	3.8	3.8	1.48	0.232
Beverage * Time	3	103.6	34.5	13.62	<0.001
Total	77	635.1	8.2		

Table 32: ANOVA for Serum Valine (mg/L)

Source	DF	SS	MS	F	p
Beverage	3	731.1	243.7	7.93	<0.001
Time	1	23.1	23.1	3.07	0.088
Beverage * Time	3	262.9	87.6	11.66	<0.001
Total	77	2352.8	30.6		

Appendix D: Institutional Review Board Proposal

Request for Approval of Research Proposal
Department of Human Nutrition, Foods, and Exercise
Virginia Tech

TITLE: The Effect of Milk on Muscle Protein Synthesis and Degradation after Resistance Exercise and on Chronic Muscle Hypertrophy

INVESTIGATOR: Janet Walberg Rankin, Ph.D.
Professor
Department of Human Nutrition, Foods, and Exercise

PURPOSE AND JUSTIFICATION:

The social ideal of the male body includes high muscularity. Thus, resistance weight training, which can result in up to 1 kg of lean weight gain per month, is popular among males of all ages. In order to accelerate muscle mass gains, many recreationally active individuals as well as athletes use dietary supplements or other dietary manipulation. For example, sales of androstenedione, a supplement that claims to increase muscle size, was estimated at $25 million in 1998 (USA Today, June 4, 1999). One survey indicated that the most frequently promoted benefit of supplements marketed to those involved in resistance weight training is muscle growth, about 60% of the products claimed this effect (Philen et al. 1992).

Research shows that resistance exercise increases protein breakdown and synthesis with a net increase in body protein. For example, protein synthesis increased 108% and proteolysis increased 51% above resting as a result of one acute resistance exercise (Biolo et al 1995). Although this suggests that protein needs are higher in resistance trainers, the value of higher protein content of the chronic diet has been controversial in its effects on lean tissue gains. While novice males performing resistance training for 8 weeks had a higher nitrogen balance if they consumed a protein supplement (compared to a carbohydrate supplement), they did not have greater lean tissue or strength gain over that period (Lemon et al. 1992).

Isotopically labeled amino acid infusion has showed that increasing blood amino acid concentration via infusion of an amino acid mixture post- resistance exercise bout caused an increase in muscle protein synthesis (Biolo et al. 1997). A recently published study from this laboratory tested whether oral ingestion of amino acids after a resistance exercise bout would have similar effects as infusion. Ingestion of a 40 grams amino acid solution over 3 hr after a strenuous resistance exercise increased net muscle protein balance (as measured by labeled phenylalanine infusion) from the negative balance noted with the placebo ingestion (Tipton et al. 1999). The authors point out that this study was "the first report of improving muscle protein anabolism in humans with an oral amino acid supplement after exercise". Thus, the benefit of amino acids on muscle protein balance existed for oral consumption as well as the earlier infusion studies. This hypothesis has not been tested with consumption of whole proteins or foods containing protein, such as milk. In addition, it is unknown whether the magnitude of the boost in muscle protein synthesis as a result of amino acid consumption after exercise will translate into superior gains in lean tissue mass and function.

In addition to measurement of protein synthesis with infusion of amino acid isotopes, molecular biology methods have been used to estimate the effect of diet and exercise interventions on muscle protein anabolism. The likely molecular mechanism for

the rapid increases observed in muscle protein synthesis following resistance exercise is translational control of protein synthesis (Booth et al. 1997). There is evidence that translation of existing mRNA may be up regulated to cause rapid changes in muscle protein synthesis in response to both dietary and exercise intervention.

Efficiency of translation is regulated by initiation factors that regulate at the level of peptide chain initiation. Several recent studies have focused on the role of eukaryotic initiation factors (eIF) in regulating translation and thus rapid changes in protein synthesis after exercise or dietary change. Yoshizawa et al. (1998) found that feeding a 20% protein diet but not a protein-free diet stimulated protein synthesis. The stimulation of protein synthesis by dietary protein was associated with changes in the binding of eIF4E to eIF4G (stimulatory complex) rather than to 4E-BP1 (inhibitory complex).

Another recent study measured the change in these complexes in rats fed different diets after exercise (Gautsch et al 1998). Rats consumed either a carbohydrate or a "complete meal" that contained protein (Ensure, Ross Laboratories) after a 2-hr run. In addition to measuring protein synthesis with a labeled leucine infusion, muscle samples were analyzed for the initiation factor complexes. They found that ingestion of the protein containing post-exercise meal but not the carbohydrate meal caused an increase in muscle protein synthesis as measured by the isotope infusion. The changes in protein synthesis were reflected by the changes in the muscle eukaryotic initiation factors: the amount of eIF4E bound to 4E-BP1 (depresses initiation of translation) was lowest for the "complete meal" while the amount of eIF4G associated with eIF4E (stimulates initiation of translation) was highest for this group.

Muscle and whole body protein breakdown has been estimated using isotopically labeled amino acid infusion paired with muscle biopsies. Biolo et al. (1997) found that infusion of amino acids after resistance exercise reduced the rate of muscle protein breakdown relative to resistance exercise in the fasted condition. Recently, a test of oral consumption of amino acids similarly increased muscle protein synthesis without the increase in muscle protein breakdown noted in the fasted condition (Tipton et al. 1999). Thus, increasing blood amino acids appears to prevent the increase in muscle protein breakdown that occurs after resistance exercise.

An important factor to consider in interpreting the above studies is that none of them used an isoenergetic comparison beverage/infusion to determine that the critical factor in reducing muscle protein breakdown is protein and not just additional energy.

Another way to examine muscle protein breakdown is by measuring total excretion of 3-methylhistidine in the urine. Several studies have been conducted that found a reduction in 3-methylhistidine excretion as a result of nutritional intervention after a resistance exercise bout. One study fed subjects carbohydrate after resistance exercise and found reduced 3 methylhistidine excretion compared to the placebo group (Roy et al 1997); they did not test a protein alone or a carbohydrate with protein mixture beverage to determine whether this would be superior to the carbohydrate drink. This study as well as work from our own laboratory (see below) that found lower 3 methylhistidine when milk was consumed after exercise suggests that post-exercise nutrient consumption may reduce acute muscle protein breakdown that occurs as a result of resistance exercise. Again, no long-term studies have been conducted to determine whether this allows greater gains in muscle mass in resistance trainers.

We (Wick et al. 1997 and *under review* for publication) performed a study to compare the effects of consumption of a milk beverage to a carbohydrate beverage or non-energetic placebo after strenuous eccentric muscle contractions that are known to cause muscle damage. The milk-based beverage consumed immediately and 2 hr

following novel eccentric resistance exercise was shown to reduce muscle protein breakdown as measured by urinary 3-methylhistidine. A limitation of this study was that no index of protein synthesis was conducted. Thus, the overall effect on protein balance could not be assessed. In addition, since this was an acute study, it is not possible to determine whether these short-term changes in protein metabolism would translate into long-term superior gains in muscle mass and function.

Statement of the Problem

In summary, consumption of foods, especially those containing protein, just after exercise may have a beneficial effect on protein synthesis and breakdown that could have a long-term effect on accretion of lean body mass in subjects participating in a resistance exercise program. Many of these studies have not had an isoenergetic comparison treatment so more research must be done to identify the ideal nutritional intervention post exercise. Although the literature does not support the value of chronically higher protein intake on lean body mass gains, the *timing of protein intake* post- exercise may be important to increase provision of amino acids, increase hormones known to be anabolic for protein, and stimulate translation of muscle protein. In other words, there might be an important *window of opportunity* for nutrition to promote muscle protein accretion. A mixture of carbohydrate with protein, such as milk, may be an optimal post-exercise beverage.

The *OVERALL OBJECTIVE* of this study is to determine whether milk has a unique value in enhancing strength and lean mass gains resulting from a resistance exercise program in young men.

SPECIFIC OBJECTIVES

Determine the acute effect of post- exercise consumption of two milk beverages (differing in protein content) on myofibrillar protein *breakdown and synthesis* following a resistance exercise workout compared to consumption of carbohydrate or a placebo.

PROCEDURES:

Subjects and Design

Forty male subjects between 18 and 25 years of age will be recruited and screened for Phase I study to determine the best milk beverage intervention to be used in Phase II. Individuals who have not participated in resistance training for at least one year will be recruited from the Virginia Tech campus. We will recruit subjects by posting fliers around campus with a brief description of the study and our phone number. Interested individuals who call will be asked several questions about contraindications of participation (including schedule, training status, allergy to milk); those who are still interested will be invited to a group session. A full description of the study will be presented with a chance for individuals to ask questions. Those who are interested in participating will be provided with an informed consent to take home. Those subjects who complete the informed consent will be given the health screening survey and those without contraindications will be considered in the selection of subjects.

Contraindications to resistance exercise including high blood pressure and other cardiovascular diseases, diabetes, orthopedic problems; contraindications to biopsies such as allergy to lidocaine; and allergy to milk will be included in the screening form (see attached health history form).

Each subject will undergo a resistance exercise test that will include two muscle biopsies and blood withdraws: baseline (fasted) and 1-hr post nutrient ingestion. The subjects will be equally divided into four groups; Placebo (NutraSweet flavored beverage), Milk1 (chocolate milk), Milk2 (skim milk flavored with chocolate powder). The beverages will be consumed immediately after the resistance exercise workout. Muscle samples will be analyzed for various eukaryotic initiation factors (eIF4E, eIF4E-eIF4G complex, and eIF4E-4E-BP1 complex) as described below. The milk beverage showing the greatest change in these factors will be used as the milk beverage during Phase II.

Acute resistance training bout:

The subject will perform five sets of eight repetitions of leg extension and leg press at 80% of 1RM (maximum strength) with 2 min between sets and 4 min between exercises (similar to that used by Tipton et al. 1999). Subjects will be provided with their beverage immediately after the workout (see below). Muscle biopsies will be performed just before starting the resistance workout and at 1 hr. Blood samples (10 mL each) will be withdrawn from an arm vein before, immediately post, and at 1-hr post exercise. The blood samples will be analyzed for insulin, growth hormone, and amino acids.

***Supplements*:**

Subjects will receive their beverage of flavored water (placebo, NutraSweet flavored drink), carbohydrate (sports beverage), Milk1 (chocolate low fat milk) or Milk2 (skim milk with flavoring such as Nestlé's Quick) immediately after their resistance-training workout. Beverages will have 5 kcal/kg as either a carbohydrate- based sports drink (1.25 g/kg of carbohydrate), chocolate low fat milk (0.92 g/kg carbohydrate, 0.21 g/kg protein, and 0.06 g/kg fat), flavored skim milk (0.875 g/kg carbohydrate and 0.375 g/kg protein). No other food will be consumed by the subjects until after the 1h post exercise biopsy.

Measurements:

Muscle biopsy - Two muscle samples (before and 1-hr post) of the vastus lateralis (thigh) will be obtained under local anesthesia from all subjects for each acute resistance test in the same leg approximately 2 cm from each other. This procedure will be done by Ms. Janet Rinehart (Certified Medical Laboratory Technician) with a physician available. Just prior to performance of the muscle biopsies, the subjects will again hear a verbal description of the procedure and be given a chance to ask questions. They will be queried again concerning any allergy to lidocaine/novocaine. A local anesthetic (lidocaine) will be injected in several punctures following cleansing. This feels like a bee sting. A half-inch wide incision is made with a scalpel blade. A 75-100 mg sample of muscle is removed using a hollow needle that is inserted through the muscle fascia. The incision is closed afterwards using steri-strips. Pressure will be immediately applied with sterile gauze and a cold pack will be placed over the incision. A pressure wrap will be put around the leg prior to the subject leaving the laboratory. They will be given instructions on how to care for the incision and warning signs of infection. They will be

provided with phone numbers to contact the experimenters or observing physician should they have any questions.

The muscle sample will be frozen in liquid nitrogen and stored for subsequent analysis of eukaryotic initiation factors: eIF4E, eIF4E-eIF4G complex, eIF4E-4E-BP1 complex using molecular biology techniques.

Urinary 3 methylhistidine and creatinine- All urine will be collected in polypropylene bottles containing hydrochloric acid has a preservative for 2 days: 24 before and after the acute resistance bout test. Samples will be stored and later analyzed for urinary 3-methylhistidine using an amino acid analyzer.

Blood analyses: Serum amino acids, glucose, insulin, growth hormone will be analyzed from samples taken before and 1 hr after the resistance exercise bout.

Appendix E: Informed Consent and Health History Questionnaire

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

TITLE: The Role of Recovery Beverages after Resistance Exercise for Active Individuals. **PHASE I.**

PRINCIPAL INVESTIGATOR: Janet Walberg Rankin, Ph.D.

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.

PROCEDURES:

General Design

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 with your legs 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 with the exercise. We will take another sample of your muscle and blood 1-hr after you complete the workout.

Specifics of measurements

Strength

About a week before the strength-training workout, we will test your maximal strength for the leg extension and leg press. This will involve warming up with a weight you think you can lift about 10 times, resting, and 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 hr 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 min. 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 hr and the steri-strips should remain on for about 3 days. You should not "baby" the leg; using is 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 2 scars 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 min.

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 140 muscle biopsies have been performed in previous studies.

Blood Samples

You will have two blood samples taken on the day of the test, one at the beginning and 1-hr after the exercise test. The amount in each sample is about 2 teaspoons.

Urine Collections

You will be asked to collect all the urine you produce 2 days, the day before the resistance exercise and the day of the resistance bout. 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. Consume all of the provided beverage after each workout session.
2. Refrain from taking any other nutritional supplements without checking first with the experimenters.
3. Give maximal effort on performance tests.
4. Come to the resistance training test and training sessions in a fasted condition (nothing to eat since the evening before).
5. Inform the experimenters if you experience any unusual symptom from any of the testing or training.
6. 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.
7. Refrain from taking aspirin for 24 hr prior to the muscle sampling procedures (to reduce chance of excess bleeding during the procedures).
8. Remain in the laboratory for at least 20 min after the muscle sampling.
9. 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.

BENEFITS OF PARTICIPATION

There is no specific benefit to you for participating in this study.

COMPENSATION

A total of $75 will be paid to you for participation in this study.

ANONYMITY 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: Tom Hurd, 231-5281

Name of Subject (please print)_____

Signature of Subject_____ Date_____

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

Name:_____ **Age:** _____ **Birth Date:** _____

Address:_____ **e-mail:** _____

Phone Numbers: Home:_____ **Work :**_____

Person to Contact in Case of an Emergency: _____

Relationship: _____ **Phone:** _____

Primary Care Physician: _____ **Phone:** _____

Medical Insurance Carrier: _____

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?	_____	_____
Date: _____		

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, short 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	____	____	_____	____

Please sign to indicate that the above information is correct:

_____ _____

_____

Print name Signature Date

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?

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

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

Drug Allergies

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

6. 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

7. 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.

Appendix F: Anthropometric Data Sheet

____/____/____

Body Weight and Anthropometric Data

Subject:_____ Age:_____

Height:_____in. _____cm. _____m.

Weight:_____lb. _____kg.

BMI

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

Skinfold Data

				Avg.
Chest	_____	_____	_____	_____
Abdomen	_____	_____	_____	_____
Thigh	_____	_____	_____	_____

Sum of Skinfolds: _____

% Body Fat: _____

Appendix G: Exercise Data Sheet

Subject:_____

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 H: Biopsy & Blood Draw Data Sheet

Subject:_____

Blood/Biopsy Recording Sheet

Date _____/_____/_____

Blood Draws

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

Biopsy

Sample	Time
Pre-Exercise	
1 Hour Post-Exercise	

Beverage Consumption

Time Started	
Time Completed	

Appendix I: Urine Volume Data Sheet

Urine Volume Data Sheet

Subject	Day 1 Volume	Day 2 Volume
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
37		
38		
39		
40		
41		
42		

Appendix J: Likert Sensory Form

Subject #_____ Date_____ Time postexercise_____

Please place an X on the line indicating your opinion on how the beverage you ingested has the qualities listed.

Tastes good

_____

terrible great!

Quenches thirst

_____

not at all completely

Fulfills hunger

_____

not at all completely

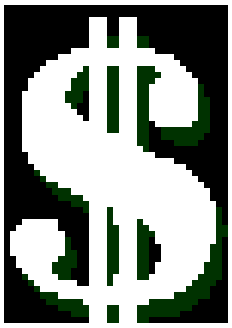
Energizing

_____

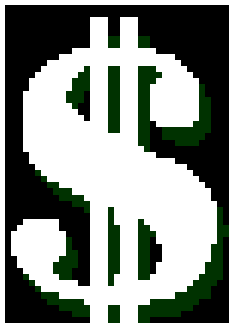
not at all completely

Appendix K: 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

Will involve:

- Total of 2 days
  (combined total of 3 hours)
- Weight lifting
- Blood samples (3)
- Muscle samples (2)
- Urine Sample

Will get:

- $75
- Strength Analysis
- Body Composition Analysis

If interested, contact Randy Bird at bird@vt.edu or Dr. Rankin at jrankin@vt.edu to see if you qualify.

Appendix L: Instructions Given to Subjects

* Given to subjects with the urine bottles for collection

Instruction for Subjects

Meat-Free Diet

On the day before testing and the day of testing, you will have to consume a meat-free diet. ***This means no hamburgers, turkey, chicken, ham, pepperoni, etc.*** You will still need to eat protein, just not from meat. So on those days, you will need to replace your meat products with foods like beans, dairy products, eggs, and peanut butter. You can have Vegeburgers if you want.

The reason for this is the measurements we are taking in your urine. We are measuring muscle protein breakdown. When you consume meat products, this alters the amount of muscle protein breakdown we see in the urine.

Urine Collection

Urine analysis will be performed on all urine voided the day of testing as well as 1 day prior (baseline measure). All urine is to be collected and turned in to the investigators during this time. There will be two 24-hr 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 War Memorial in the morning when you come for testing and in the morning the day after testing.

Stop eating by 8:00PM on the night before testing. Do not eat anything on the day of testing until after you leave the laboratory. Arrive at War Memorial upstairs in room 230 at 7:50AM on the day of testing.

Any Questions? Randy Bird: 953-2610

* Given to subjects after the second muscle biopsy.

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 hr 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

Randy Bird: 953-2610, 231-7708
Dr. Julie Neely, MD: 951-7236
Ms. Janet Rinehart: 231-2667, 953-0672
Dr. William Epstein, MD: 951-7407
Dr. Janet Rankin, PhD: 231-6355, 552-9017
University Student Health Services: 231-6444

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Vita

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