THE EFFECT OF CREATINE SUPPLEMENTATION ON MUSCLE FUEL STORES, BODY COMPOSITION, AND EXERCISE PERFORMANCE DURING ENERGY RESTRICTION

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ABSTRACT

The purpose of this investigation was to determine the effects of a four day creatine load and simultaneous energy restriction on muscle creatine content, exercise performance, and body composition in 24 male recreational resistance trainers, age 18-26. Sixteen subjects were randomly divided into placebo (Pl, n=8) and creatine supplement (CrS, n=8) groups. Control (C, n=8) subjects of the same age were recruited separately $g \cdot d^{-1}$ to complete the performance and body composition tests while consuming their normal diet. The CrS group was administered 20 $g \cdot d^{-1}$ of creatine monohydrate (Cr) mixed with 5 $g \cdot d^{-1}$ of sucrose, while the Pl group was administered 25 of sucrose. Both CrS and Pl consumed a formula diet of 75.3 kJ (18 kcal) $\cdot kg^{-1} \cdot d^{-1}$ for 4 d. Testing before and after energy restriction consisted of a repeated sprint cycle performance test (10 sprints of 6s, with 30s rest), hydrostatic weighing, and resting needle muscle biopsy. Testing revealed that subjects in CrS and Pl demonstrated significant decreases in body weight and % body fat (%BF) with no difference between groups. However, Pl demonstrated a significantly greater % loss in FFM ($2.4 \pm 0.25\%$) compared to CrS ($1.4 \pm 0.4\%$) ($p<0.05$). The muscle fuel stores of CrS and Pl responded significantly to the diet. Significant increases in muscle total Cr ($p<0.01$), free Cr ($p<0.01$), and CrP ($p<0.05$) of 16.5%, 16.8%, and 16% respectively were demonstrated by CrS over the energy restriction period, while Pl demonstrated significant decreases of 7.2% and 8.2% respectively in muscle total Cr ($p<0.01$) and free Cr ($p<0.05$). There were no significant differences between groups for performance during the cycle test, however, there were trends toward group by time interactions for performance enhancement in CrS relative to Pl, as total work ($p=0.078$) and work capacity ($p=0.058$) increased $3.8 \pm 2.2\%$ in CrS and decreased $0.5 \pm 0.4\%$ in Pl.

It was concluded that short-term energy restriction resulted in decreased muscle Cr storage, and that Cr supplementation during energy restriction increased muscle Cr and CrP stores. Consumption of Cr allowed CrS to lose a significantly lower % FFM compared to Pl. Cr supplementation resulted in trends toward improved performance in CrS relative to Pl after energy restriction, but did not influence losses in body weight or %BF.

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CHAPTER I INTRODUCTION

The widespread supplementation of creatine (Cr) monohydrate by athletes in many sports hoping to improve anaerobic exercise performance has led to a need for research into the benefits of Cr loading as well as potential side effects and limitations of this practice. The mechanism by which Cr monohydrate supplementation improves anaerobic performance may be an increase in muscle Cr and creatine phosphate (CrP). Cr and CrP are compounds that are important to energy production during exercise, especially at high exercise intensities.

Many sports require short periods of high or maximal intensity exercise with intermittent periods of rest. Athletes competing in these sports may gain from an increase in the availability of CrP in the muscle. Wrestlers and resistance trainers, as well as football, basketball, soccer, tennis, hockey, and lacrosse players are well trained in this pattern of exercise. Many cyclists, swimmers, and runners utilize interval training as a method of increasing the amount of time spent at high intensity within a workout. Finally, weight training is a method of building strength and power that is utilized by athletes in virtually all sports.

Athletes in many of these sports are at an advantage when they maximize strength and power while also minimizing body weight. Since a lack of energy fuels can limit performance, athletes that restrict calories may be risking decrements in performance. It is difficult to determine at what point an advantage is gained as a result of lowering body weight, and when that advantage is lost due to performance deficits. Indeed, the practice of weight loss is often taken to extremes, and recently has resulted in death for several college wrestlers who were cutting weight in preparation for competition. Recreational athletes often exercise in hopes of losing weight or to avoid becoming overweight. If they combine their exercise with a low calorie diet, they may find it difficult to continue to exercise at a high intensity, since their energy deficit limits their ability to exercise.

Creatine may be helpful to these athletes. Athletes attempting to lose body weight or body fat often combine energy restriction and exercise to achieve their goal. Supplementation with Cr during this period of restricted calories and increased exercise

may allow for the maintenance or improvement of performance combined with losses in body weight and body fat.

Statement of the Problem

Athletes often restrict energy intake in preparation for athletic competition, with the goal of losing body weight while maintaining lean body mass. In sports such as running, low body weight may reduce the energy cost to the individual. In aesthetic sports like dancing, figure skating, or bodybuilding, decreased fat mass helps enhance the appearance of the athlete. In wrestling, energy restriction is used to attain a weight class lower than the individual's normal body weight.

Optimal anaerobic exercise performance is dependent on the ability of the body to quickly produce energy in the form of adenosine triphosphate (ATP). The energy held in ATP becomes available when the high-energy bonds between phosphate groups are broken, releasing a phosphate ion and producing adenosine diphosphate (ADP). Anaerobic exercise performance may be impaired by energy restriction due to reduced ability to regenerate ATP as a result of impaired glycolytic function and decreased muscle Cr and CrP levels.

Studies using obese humans and rats have shown that energy restriction results in decreased muscle CrP levels. It is also known that energy restriction can impair performance due to reduced energy stores and dehydration. Thus, it is reasonable to hypothesize that supplementation during energy restriction will help maintain muscle Cr stores and exercise performance.

Athletes of all types may benefit from creatine supplementation at some point in their training schedule, and athletes of many types engage in weight loss practices. Since creatine supplementation may negate some of the negative effects of weight loss, this research will have implications for athletes who want to restrict energy to lose body weight or change body composition while maintaining performance levels.

Objectives

- To determine whether Cr and CrP stores decrease due to energy restriction.

- To determine if creatine supplementation will affect muscle creatine or CrP levels during energy restriction.
- To determine if creatine supplementation will affect anaerobic exercise performance during energy restriction.
- To determine if creatine supplementation will affect body composition during energy restriction.

Hypotheses

H_0: Muscle Cr, CrP, and ATP stores of male resistance trainers are not affected during four days of energy restriction at 18 kcal/kg/day.

H_0: Cr supplementation with 20 g/day for four days will have no effect compared to a placebo on muscle Cr, CrP, and ATP stores in male subjects during four days of energy restriction at 18 kcal/kg/day.

H_0: Cr supplementation with 20 g/day for four days will have no effect compared to a placebo on repeated sprint cycle performance in male subjects during four days of energy restriction at 18 kcal/kg/day.

H_0: Cr supplementation with 20 g/day for four days will have no effect compared to a placebo on body composition in male subjects during four days of energy restriction at 18 kcal/kg/day.

Delimitations

- The subjects were trained, healthy male recreational resistance trainers age 18-26 with no history of steroid use.
- All subjects abstained from supplementing with Cr at least 30 days prior to the study.
- Performance was measured during repeated sprints on a cycle ergometer.
- Body weight was measured two weeks prior to the first performance test in order to ensure that all subjects were weight stable.
- The energy restriction diet was a formula diet (Ensure, Ross Laboratories) consisting of 18 kcal/kg/day for 4 days with 54.7% carbohydrate, 21.3% protein, and 24% fat.

- The independent variables were supplementation with 5 g creatine monohydrate plus 1 g of sucrose, 4 times per day for the creatine group and 6 g of sucrose for the placebo group. The control group did not undergo energy restriction or supplementation during the study.
- The dependent measures were: muscle Cr, CrP, and ATP concentrations (creatine and placebo groups only), percent body fat, fat free mass, total work performed, peak power, work capacity, maximal power, peak power fatigue index and total work fatigue index during the sprint cycle performance test (all groups).

Limitations

- The sprint cycle ergometer test may have been unfamiliar to the subjects. The two practice tests may not have been enough to completely familiarize the subjects and eliminate the learning effect during the performance tests.
- The content of the formula diet may not have been representative of the subjects' normal diet. For instance, the formula diet contained no creatine.
- Diet records were not taken. The formula diet was based on body weight for all subjects, not as a percentage of the normal food intake of the subjects.
- The creatine load of 4 days is shorter than the 5 day loading period that is often recommended.
- The weight maintenance period of 2 weeks may not have been long enough to overcome changes if the subjects had previously lost weight.
- The control group was not randomly assigned.

Definitions and Symbols

- **Cr** Creatine
- **CrP** Creatine Phosphate
- **ATP** Adenosine Triphosphate
- **ADP** Adenosine Diphosphate
- **CPK** Creatine Phosphokinase
- **FFM** Fat-free Mass

- **%BF** Percent Body Fat
- **NMR** Nuclear Magnetic Resonance
- **RM** Repetitions Maximum. In weight lifting, the maximal amount of weight that can be used in an exercise for a certain number of repetitions.
- **Aerobic** In the presence of oxygen. Oxidative metabolism
- **Anaerobic** In the absence of oxygen
- **Phosphagen System** The use of CrP to replenish ATP from ADP during exercise, anaerobically.
- **Glycolysis** The metabolic pathway beginning with glucose-6-phosphate and ending with pyruvate, yielding 2 ATP per glucose molecule. Under anaerobic conditions, pyruvate forms lactate. Aerobically, pyruvate forms acetyl coA and enters the TCA cycle.
- **Glycogenolysis** The hydrolysis of glycogen in the liver or muscle to its constituent units of glucose-1-phosphate or glucose.
- **Mitochondria** Organelle within the cytosol of cells. The site of aerobic ATP production.
- **Creatine Group (CrS)** The group of subjects (n=8) who received 5 g of creatine monohydrate plus 1 g of sucrose 4 times per day during the 4 day energy restriction period.
- **Placebo Group (Pl)** The group of subjects (n=8) who received 6 g of sucrose 4 times per day during the 4 day energy restriction period.
- **Control Group (C)** The group of subjects (n=8) who did not undergo energy restriction and did not receive any supplement. The main purpose of the control group was to control for the effects of repeated testing on the sprint cycle ergometer tests.

Basic Assumptions

- It was assumed that all subjects stopped supplementing with creatine at least 30 days prior to the first weight measurement.
- It was assumed that all subjects were truthful in their reporting of no anabolic steroid use.
- It was assumed that all subjects adhered to the formula diet and consumed no other calories than those supplied by the researchers.
- It was assumed that all subjects gave a maximum effort for the repeated sprint cycle performance test.
- It was assumed that all subjects fasted for 12 hours prior to the performance and body composition tests.

Summary

Research showing depression of muscle CrP and impaired performance during energy restriction is important to competitors in sports where energy restriction is common. A decrease in muscle Cr or CrP may be problematic for athletes engaging in exercise of short duration and high intensity, such as weightlifting and sprint cycling. Creatine supplementation has been shown to increase muscle Cr and CrP levels and to result in improved performance during repeated bouts of brief, high-intensity exercise. Creatine supplementation may allow for maintenance of muscle Cr and CrP, and performance during energy restriction.

Energy restriction has also been shown to result in losses in body fat and FFM. Creatine may play a role in enhancing muscle protein synthesis. Supplementation with Cr during energy restriction may allow for the desired loss of body fat coupled with maintenance of or minimization of the loss of FFM.

The performance, muscle Cr and CrP stores, and body composition benefits associated with Cr supplementation have not been examined in athletes during energy restriction. It is hypothesized that Cr supplementation will help maintain muscle Cr and CrP levels and will reduce FFM losses and performance decrements due to energy restriction.

CHAPTER II REVIEW OF THE LITERATURE

Introduction

Energy is provided within the body by the removal of a phosphate group from adenosine triphosphate (ATP), forming adenosine diphosphate (ADP). The bonds between phosphate groups are termed high energy bonds, and when these bonds are broken, energy is released which can be used to perform work, such as in muscle contraction. A small amount of ATP (about 24 mmol/kg dry muscle) is available within the cytosol of the cell at the onset of exercise, but contains only enough energy to fuel exercise for about two seconds (s) (21). Clearly, ATP must be replenished virtually simultaneous to the onset of exercise. In fact, muscle ATP concentrations do not change with the onset of exercise, because ATP is regenerated rapidly. The phosphagen system is the most rapid source of ATP regeneration after the onset of exercise. Creatine phosphate donates its phosphate to ADP, thereby replenishing the cell with ATP, in a reaction catalyzed by the creatine phosphokinase (CPK) enzyme. In a reverse reaction also catalyzed by CPK, Cr is phosphorylated by the removal of a phosphate from ATP, forming ADP and CrP (Fig. 1). Since Cr is the precursor to CrP, increased Cr levels may allow regeneration of CrP to occur more rapidly (19). Also,

$$\mathbf{CrP + ADP + H^{+} \quad \Leftarrow CPK \Rightarrow \quad Cr + ATP}$$

Fig. 1 The Creatine Phosphokinase Reaction

elevated muscle Cr and CrP may act as a buffer that maintains intracellular pH during exercise (19) and stimulate muscle protein synthesis and hypertrophy (18,30,55).

Creatine Metabolism During High-Intensity Exercise

During high-intensity exercise, the energy demand is greater than can be provided aerobically. The phosphagen system is a major anaerobic energy pathway, and therefore is a source of ATP during high-intensity exercise. Research regarding the physiological

responses to high-intensity exercise has shown that Cr and CrP play a major role in providing energy, especially during the initial 10 s of exercise (4,15,44,49,52). Further, during maximal exercise, the maximal rate of ATP production from CrP is attained within the first 2 s of exercise (21,49).

Once CrP phosphorylates ADP to ATP, the resulting free Cr enters the mitochondria, where CrP is resynthesized (21). The resynthesis of CrP by mitochondrial CPK is dependent upon the aerobic process of oxidative phosphorylation. This reliance on aerobic metabolism is thought to be a reason for the rapid decline in ATP production from CrP during maximal exercise.

CrP is not the only source of ATP during high-intensity exercise of less than 30 s (15,21,49). Glycogenolysis is the breakdown of glycogen to individual units of glucose-1-phosphate. Glycogen derived glucose-1-phosphate or glucose from the bloodstream enters the glycolysis pathway, which is also an important source of anaerobic energy. When oxygen is limited, such as during high-intensity exercise, pyruvate forms lactic acid. CrP degradation and glycogenolysis begin simultaneously with the onset of exercise (49). Glycolysis, however, does not reach its maximal rate of ATP production until 5 s (49). Once this maximal rate is achieved, anaerobic ATP production from glycolysis is maintained for 15 to 20 s of exercise, in contrast to the phosphagen system, where ATP production peaks at 2 s, then rapidly declines (21).

Serresse et al. (44) estimated the contributions of the various energy systems during high-intensity, short duration exercise. Twenty five male subjects age 17 to 24 performed 10, 30, and 90 s maximal cycle ergometer sprints separated by 10 minutes of rest. During the 10 s sprint, the contribution of the phosphagen system to ATP production was estimated to be 53%, and anaerobic glycolysis and the aerobic system contributed 44% and 3%. During the 30 s sprint, the contribution of CrP to ATP production was estimated to be 23%, while glycolysis and aerobic metabolism provided 49% and 28%, respectively. Estimations of ATP contributions during the 90 s sprint were 12%, 42%, and 46% for CrP, anaerobic glycolysis, and aerobic metabolism respectively.

Gaitanos et al. (15) studied the contributions of the various energy systems in male subjects during 10 seated 6 s sprints with 30 s rest on a cycle ergometer. All subjects

performed a prescribed warm-up and 5 minutes of stretching before the repeated sprint performance test. This warm-up protocol was chosen because it causes only minor metabolic disturbances. Muscle biopsies were taken from the vastus lateralis before warm-up, after sprint 1, 10 s prior to sprint 10, and after sprint 10. Blood was drawn before warm-up, after sprint 1, after sprint 5, after sprint 9, after sprint 10, and 3, 5, and 10 minutes after exercise.

Mean power output was decreased by 26.6% after 10 sprints, while peak power output decreased by 33.4%. The time to peak power in all sprints was within 2 s of onset of exercise. Blood lactate reached its maximum of 12.6 +/- 1.5 mmol/l after sprint 9 and did not change through 10 minutes of recovery. After sprint 1, muscle CrP concentration decreased 57%, and muscle glycogen decreased 14%. After sprint 10, muscle CrP was 16%, while muscle glycogen was 63% of resting value. It is important to note that muscle glycogen dropped 6% during the tenth sprint, while CrP dropped 35% (15). Of the anaerobic ATP production during the first sprint, 49.6%, or 44.3 mmol/kg dry muscle (dm) came from CrP, and 44.1% or 39.4 mmol/kg dm came from glycolysis, with the remaining 6.3% from free ATP. During the last sprint, 80.1% of anaerobic ATP production, or 25.3 mmol/kg dm, came from CrP, while 16.1%, or 5.1 mmol/kg dm, came from glycolysis. Overall, anaerobic ATP production fell from 89.3 mmol/kg dm during sprint 1 to 31.4 mmol/kg dm during sprint 10, a decrease of 35% (15).

The energy used during the first sprint was derived mainly from anaerobic sources. During the last sprint, the anaerobic ATP production was 35% of that during sprint 1, but average power output was still 73% of sprint 1. It was concluded that during sprint 10, the main sources of ATP were the phosphagen system and oxidative metabolism, with anaerobic glycolysis contributing a small amount of energy. This study showed that the absolute contributions of anaerobic glycolysis and the phosphagen system to energy production during repeated 10 s bouts of high-intensity exercise diminishes in later bouts. However, the relative contribution of energy derived from anaerobic glycolysis diminishes substantially, while that of the phosphagen system increases. Thus, performance during repeated sprint exercise may benefit from oral Cr supplementation, while performance during single effort sprints may be unaffected.

Trump et al. (52) investigated the contribution of CrP to energy production in male subjects during three 30 s bouts of maximal cycling separated by 4 minutes of rest. By occluding the thigh of one leg with a cuff after bout 2, CrP resynthesis was prevented due to a lack of blood flow. Bout 3 was then performed with blood flow restored. Muscle biopsies were taken from the vastus lateralis before and after bout 3. The lack of CrP resynthesis in the cuffed leg allowed for an estimate of the relative importance of CrP to sustaining performance during the third sprint, through a comparison of performance with and without the availability of CrP.

Prior to bout 3, average CrP concentration in the muscle was reduced to 20.7 mmol/kg dm in the cuffed leg, compared to 63 mmol/kg dm in the control leg, showing that the cuff inhibited CrP resynthesis. During bout 3, total work was reduced by 15% in the cuffed leg compared to the control leg, whereas total work had been similar in the previous bouts. The contribution of glycolysis was 10-15% in both legs. The reduction in power in the cuffed leg was mostly manifested within the first 15 s. CrP concentration decreased 3.1 mmol/kg dm in the cuffed leg and 47.5 mmol/kg dm in the control leg. It was concluded that CrP provided about 15% of ATP production during the third bout, while the majority of the energy provision (70%) was derived from oxidative metabolism. Similar to the study by Gaitanos et al. (15), Trump et al. (52) showed that the aerobic metabolism and the phosphagen system contributes a large amount of energy to the latter bouts of repeated high-intensity exercise.

Single exercise bouts of maximal effort lasting less than 10 s rely on CrP for the majority of ATP production. During high-intensity, intermittent exercise, other energy sources become important, and the ability to recover sufficiently between exercise bouts is essential to the maintenance of performance. However, if recovery time between exercise bouts is insufficient, performance will diminish in successive bouts. Four minutes appears to be enough for full restoration of muscle ATP and CrP concentrations (49). It has been shown that repeated exercise bouts of 30 s separated by 4 min of rest result in 60% losses in total work (49,52). The power loss is a result of decreases in anaerobic glycolysis, while CrP contributes an equal amount of ATP to each successive sprint, and aerobic metabolism increases. When the rest period is as low as 30 s, CrP is not fully

resynthesized. Thus, in later sprints, the absolute contribution of glycolysis and CrP to ATP production diminishes, and that of aerobic metabolism increases.

The Phosphocreatine Energy Shuttle

Creatine and CrP also have important functions during endurance exercise (12,56). During endurance exercise, the majority of ATP is produced in the mitochondria by aerobic metabolism. After CrP is hydrolyzed to Cr, it is rephosphorylated in the mitochondria by oxidative metabolism, in a reaction with a ½ life of ~ 30 s (56). It was formerly thought that ATP produced in the mitochondria by oxidative phosphorylation diffused to the myofibrils for hydrolysis and energy under aerobic conditions (5). However, this theory did not explain some characteristics of the system, such as the presence of CPK isoenzymes at both the mitochondria and the myofibrils, the stimulation of mitochondrial respiration by Cr, and the compartmentation of ATP and ADP resulting in a loss of muscle contraction even without a total loss of ATP. The phosphocreatine energy shuttle has been proposed as a more likely system of transporting ATP from the mitochondria to the myofibrils than diffusion (5).

Under this model, the ATP produced by oxidative phosphorylation through the electron transport chain is used to phosphorylate Cr in the mitochondria. The CrP energy shuttle then transfers the high energy phosphates from the mitochondria to the cytosol to phosphorylate ADP at the site of utilization, the myofibrils. The resulting free Cr is shuttled back into the mitochondria to repeat the process (12,56). The liberation of Cr at the myofibrils is the signal for the activation of the shuttle (5).

CPK appears to function in maintaining the concentration of ATP available locally at many sites of ATP utilization (5). CPK concentrations are high in skeletal muscle sarcoplasmic reticulum, in close proximity to the Ca^{2+} ATPase, where the CPK helps to maintain a high rate of Ca^{2+} uptake. CPK concentrations are also high in the sarcolemma, in the ribosomes, and in the plasma membrane, where a high ATP:ADP ratio is important for ion transport by the Na^{+}-K^{+} ATPase. At these sites, and at the myofibrils, the phosphocreatine energy shuttle provides CrP for the production of ATP from locally produced ADP.

Dietary Influences on Muscle Creatine

Creatine is synthesized endogenously from arginine and glycine in the liver and kidneys, in a two step reaction; the second step is a methylation involving S-adenosyl methionine. It can also be consumed in the diet, from meat and fish. Ninety five percent of body Cr is found in the muscle, with the remainder located in the liver, kidneys, brain and testicles (25). The majority (60-70%) of muscle total Cr is found in the phosphorylated form as CrP, which can not pass through the plasma membrane (56). Endogenous synthesis and dietary intake provides about 2 g of Cr per day, which replaces the amount of Cr broken down to creatinine and excreted.

Vegetarians consume a minimal amount of Cr, since their diets do not contain muscle foods. As a result, the only source of Cr for a vegetarian is endogenous synthesis (9). Vegetarians exhibit a low excretion rate of creatinine, as well as low plasma Cr. There is evidence that vegetarians have normal muscle Cr stores (25)

Other aspects of the diet than Cr content can affect the metabolism of Cr. Green et al. (17) found that carbohydrate ingestion can increase muscle Cr accumulation during supplementation. Twenty four healthy men were given either 5 g of Cr 4 times a day, or 5 g of Cr 4 times a day followed 30 minutes later by 93 g of simple carbohydrate. Muscle biopsies were taken before and after the 5 day loading period. The total Cr content of subjects who ingested Cr plus carbohydrate was 60% more than subjects that ingested Cr alone. Further, subjects consuming Cr and carbohydrate together displayed a significant decrease in urinary creatinine. It was proposed that the elevation in insulin as a result of carbohydrate ingestion modulated the storage of Cr.

Vandenberghe et al. (54) tested the effects of caffeine ingestion simultaneous to Cr consumption during a Cr load of 6 days. Muscle Cr levels increased for both the Cr supplemented group and the Cr plus caffeine group, but remained unchanged in the Placebo group. Performance in a knee extensor exercise test was improved in the Cr supplemented group but not in Cr plus caffeine group. It was concluded that caffeine ingestion simultaneous with Cr ingestion results in a negation of potential performance enhancement from increased muscle Cr levels.

Supplementation and Muscle Creatine

It has long been known that Cr in the body was related to the Cr content in the diet (1). Yet, the determination that increasing dietary Cr could dramatically increase muscle Cr was not made for many years. First, it was necessary to determine normal muscle Cr levels in man. Harris et al. (24) used the needle muscle biopsy technique to collect muscle samples from the quadriceps femoris muscles of 81 male and female subjects, age 18 to 30. The mean total Cr content of the subjects was 124.4 +/- 11.21 mmol/kg dry weight (dm). The mean free Cr content was 49.0 +/- 7.62 mmol/kg dm, while the mean CrP content was 75.5 +/- 7.63 mmol/kg.

Harris et al. (25) first investigated the question of whether supplemental Cr ingestion would result in elevated muscle Cr levels. The authors performed preliminary work to determine a suitable supplementation dose. Since Cr uptake is a saturable process in rats, the authors sought a dose that would increase the plasma Cr concentration above 500 umol/L. Creatine doses of 1g or less were found to result in slight increases in plasma Cr that rarely exceeded 100 umol/L. Doses of 5 g, similar to the amount of Cr in 1 kg of uncooked steak, resulted in a mean peak plasma Cr concentration of 795 umol/L after 1 hour.

The 5 g dosage of Cr was chosen, and when ingested 4 to 6 times per day for 2 to 6 days, resulted in elevations in muscle Cr content of about 20% in the quadriceps femoris of 17 subjects. The mean muscle Cr concentration before and after supplementation was 126.8 and 148.6 mmol/kg dm respectively, and both Cr and CrP contributed to the increase in total Cr composition. It was noted that those subjects with the lowest initial Cr levels had the most pronounced elevation in muscle Cr.

Greenhaff et al. (19) measured muscle Cr stores in 8 subjects by muscle biopsy before and after Cr supplementation of 26 g/day for 6 days. Three subjects did not respond to the Cr supplementation with increases in muscle Cr. However, the 5 that began supplementation with the lowest muscle Cr levels had the largest increases in total muscle Cr. The authors concluded that some individuals are responders to Cr supplementation while others are nonresponders.

In addition, exercise may influence the change in muscle Cr following supplementation. Five subjects in the Harris et al. (25) study performed 1 hour of hard exercise with 1 leg per day of supplementation. Elevations in muscle Cr concentration were greater in the exercised leg (162.2 mmol/kg dm) compared to the control leg (148.5 mmol/kg dm), although both were increased relative to baseline (118.1 mmol/kg dm). The authors concluded that oral supplementation of 20 to 30 g of Cr results in significant increases in muscle Cr content, and that exercise enhances the uptake of Cr into the muscle, perhaps due to increased blood flow to the muscle or enhanced cellular Cr transport (25).

Hultman et al. (29) aimed to characterize the rise and fall in muscle Cr before and after supplementation, to determine if muscle Cr levels could be maintained after elevation by ingesting Cr at a rate similar to the degradation of Cr to creatinine, and to compare low-dose Cr supplementation with the dose used by Harris et al. (25). After supplementing with 5 g of Cr 4 times per day for 6 days, 6 subjects ceased supplementation, and underwent muscle biopsies at days 7, 21, and 35. Muscle Cr stores were increased by a mean of 20%, from a mean of 123.4 mmol/kg dm to 145.1 mmol/kg dm, and most of the increases in muscle Cr were due to increases in free Cr rather than CrP. The cessation of Cr supplementation resulted in a gradual decline in muscle Cr concentration over 30 days to return to presupplementation levels.

Nine subjects continued supplementation after the initial 6 day period, but thereafter ingested 2 g/day for 28 days, rather than the initial 5 g/day (29). These subjects also underwent muscle biopsies at days 7, 21, and 35. A dose of 2 g/day was chosen because it represents the rate of Cr degradation to creatinine. The continuation of supplementation at 2 g/day resulted in the maintenance of muscle Cr concentrations. Nine subjects ingested Cr at a rate of 3 g per day for 28 days, and underwent muscle biopsies before supplementation, and on days 15 and 29. The low-dose of Cr over 28 days resulted in muscle Cr elevations similar to those seen over 6 days at the higher dose of 20 g/day (121.8 mmol/kg dm to 142.0 mmol/kg dm). It was concluded that ingesting 20 g of Cr for 6 days is a rapid way to load the muscles with Cr, that this elevated Cr concentration can be maintained by ingesting 2 g/day thereafter, that muscle Cr will

gradually return to baseline levels if supplementation is ceased, and that ingesting 3 g of Cr per day for 28 days will result in similarly elevated muscle Cr levels. The authors also reported that Cr supplementation at the higher dose for 6 days resulted in no further increases in muscle Cr than were seen at 5 days.

As a result of the studies by Harris et al. (25) and Hultman et al. (29), studies regarding the effects of short-term Cr supplementation generally prescribe supplementation periods of 4 to 6 days, and Cr doses of 5 g, 4 times per day. A study by Noonan et al. (37) determined the supplementation dosage for each subject based on body weight. Those subjects that consumed 100 mg per kg per day for 8 weeks during a weight training and speed conditioning period improved performance in the 40 yard dash, while subjects consuming 300 mg per kg per day and controls did not. Subjects that consumed 100 mg per kg per day and 300 mg per kg per day improved 1 RM bench press, but those consuming 300 mg per kg per day had a greater improvement. Unfortunately, muscle Cr concentrations were not measured, and ½ of the subjects had the flu during the post test, which may have affected performance due to sickness and/or dehydration. The evidence that different dosages resulted in varied performance effects shows that it would be beneficial to explore the possibility that basing supplement dosages on body weight or fat-free mass may result in a pattern of muscle Cr elevation different from that which has been reported previously.

Although Cr supplementation may not be effective in elevating muscle Cr stores in all individuals, it is apparent that the ingestion of 20 g of Cr per day for ≥ 4 days may have a beneficial effect on muscle Cr status. Those people that have naturally high muscle Cr levels may not benefit from Cr supplementation. For example, athletes may maximize their Cr stores as a result of training. Also, there may be an optimal level of supplementation that would minimize the loss of Cr to the urine while still maximizing muscle Cr stores over short term that has yet to be determined. The high cost of Cr supplements could then be alleviated somewhat as a result of maximizing supplementation efficiency.

Creatine Supplementation and Body Composition

A number of studies have found a body mass increase of 1 to 2 kg during Cr supplementation for 5 to 7 days with 20 to 30 g Cr per day, (2,10,19,34,47,53,55), while a few studies have found no effect of Cr supplementation on body mass (22,41,50,51). Several studies concerning Cr supplementation have incorporated body composition assessment to determine the composition of the weight gain (10,22,33,34,38).

Earnest et al. (10) measured body mass and body composition in 8 strength trained athletes before and after supplementing 20 g of Cr or placebo per day for 28 days. There was a significant body mass increase of 1.7 kg in the creatine group, whereas the placebo group did not demonstrate a change in body mass. There was also a non-significant increase in fat-free mass of 1.6 kg ($p=0.054$), while the placebo group demonstrated no change in fat-free mass. There was no change in body fat in either group. The use of a small number of subjects in this study may have decreased the statistical power and caused the change in fat free mass to be insignificant.

Noonan et al. (38) measured the effects of varying doses of Cr supplemented for 8 weeks on body mass, percent body fat, and fat-free mass in 39 male college football players. No significant differences in body mass, percent body fat, or fat-free mass were observed between groups. However, both the 100 mg/kg and 300 mg/kg Cr supplementation groups had significant increases in fat free mass, and the 300 mg/kg group had a significant increase in body mass after supplementation. As noted previously, ½ of the subjects in the study developed the flu. This could have had a significant effect on body weight changes. Therefore, the results of this study should be viewed with caution.

Kelly et al. (33) measured body mass, lean body mass and body fat in 18 male powerlifters before and after a 5 d supplementation period with either 20 g/day of Cr or placebo, followed by 21 d of supplementation with 5 g/day. Subjects that supplemented with Cr had a significant mean increase in body mass of 2.8 kg while there was no increase in body mass in the placebo group. Since there was no significant change in percent body fat in either group, lean body mass was found to increase in the Cr group at a significance level similar to that for changes in body mass.

Terrillion et al. (50) measured body weight prior to two 700 m runs separated by 7 d, during which match-paired subjects supplemented with 20 g/day of Cr or placebo. There was no significant change in body mass in either group.

Increases in body mass have been observed in some studies as a result of supplementing with Cr for periods of 4 days to 8 weeks. The initial change in body weight is rapid, corresponding to the rapid increases in muscle Cr content observed during supplementation (25). When supplementation is continued longer than a 4 to 6 day period, changes in muscle Cr content are minimal. Further changes in body mass or body composition may result from increases in training volume causing increases in muscle mass. Body composition assessment during supplementation periods of varying lengths of time indicate that these increases in body mass are not due to increases in body fat, but rather from increases in lean body mass.

It has been proposed that the increased body mass and lean body mass associated with Cr supplementation may be due to total body water retention (2,18,29). A few studies have measured water retention as part of their body composition assessment. Kreider et al. (34) used a dual X-ray absorptiometer (DEXA) to measure body mass, body composition, and total body water before and after 28 days of supplementation with placebo or 15.75 g of Cr per day in 25 football players. The Cr group was found to have greater increases in body mass (2.2 kg compared to 0.8 kg) and fat/bone-free mass compared to the placebo group. There were no differences between groups in mean body fat or % body water changes.

Grindstaff et al. (22) measured body composition in 18 competitive male and female amateur swimmers before and after 9 days of supplementation with either 21 g of Cr or placebo. No significant changes were found in total body weight, fat-free mass, fat mass, percent body fat, or total body water. Since there was no increase in body weight, an increase in fat-free mass or total body water was not expected. The lack of changes in body weight may indicate that the supplementation protocol did not have an effect. Since muscle Cr content was not measured, it is not possible to determine if muscle Cr content was affected. Therefore, the literature at this time does not support the theory that water retention within the muscles due to increased muscle Cr content is the cause of the

increase in body mass associated with Cr supplementation. Thus far, there has been no published research looking at changes in intracellular water with Cr supplementation.

The increases in body mass resulting from Cr supplementation may be due to increased muscle fiber size and protein synthesis as well. Studies have shown that Cr may be involved in the stimulation of protein synthesis. Ingwall et al. (30) showed that Cr may be the signal which causes hypertrophy following exercise since increased muscle Cr increases protein synthesis in vitro. Bessman et al. (3) theorized that the phosphocreatine energy shuttle may be involved in muscle hypertrophy. During and after exercise, the phosphocreatine energy shuttle is especially active, as it functions to maintain ATP concentrations within the cytosol and deliver energy to working muscles. At this time, the shuttle also supplies greater amounts of energy to the ribosomes. With a greater availability of ATP as a result of exercise induced activity of the phosphocreatine energy shuttle, the ribosomes are able to increase protein synthesis, resulting in muscle hypertrophy.

Increased cellular volume due to water retention is thought to be an important mechanism involved in the regulation of ion transporters and ion channels in the cell membrane, and may be related to antiproteolytic effects of the hormone insulin and the amino acid glutamine (56). Cellular swelling as a result of elevated muscle Cr may have an anabolic effect increasing protein synthesis, resulting in increases in fat free mass (26). Nonetheless, it has yet to be shown that Cr supplementation results in increases in water retention or increased cellular volume.

Short Term Energy Restriction and Body Composition in Athletes

There are several motivations for an athlete to undergo energy restriction. Often, the goal is to reduce body fat and maintain lean body mass. Depending on the sport and the situation, the energy restriction period can be long or short. Runners may want to maintain low body weight to reduce the energy cost of performance. In diving, figure skating, and gymnastics aesthetic appearance is important, so athletes attempt to maintain low body fat throughout the season. In bodybuilding, the goal is large muscle mass combined with very low body fat. In wrestling and weightlifting, a dramatic short-term

energy restriction period combined with intense training serves to reduce body weight in an attempt to achieve a low weight class.

Energy restriction can have significant effects on body weight and body composition. Several studies have measured declines in FFM in bodybuilders as well as wrestlers as a result of their weight loss efforts (27,35,57). These athletes are likely to combine resistance training and relatively high protein intake with energy restriction to mediate body composition changes during energy restriction. It is expected that resistance exercise and high protein diet will stimulate the synthesis of muscle protein, and combining these strategies during energy restriction may attenuate the associated loss of lean body mass while allowing fat loss to continue (57).

Heyward et al. (27) measured lean body mass changes in 9 male and 12 female bodybuilders 6 to 17 weeks before and just prior to competition. Seventy five percent of the subjects lost lean tissue during the energy restriction period, and the mean loss in lean body mass was 1.3 kg. Consequently, it was concluded that substantial resistance training during energy restriction may not prevent lean tissue loss.

Melby et al. (35) measured body composition changes due to weight-cycling in 12 collegiate wrestlers over the course of a season. Hydrostatic weighing was performed before the season, 24 to 48 hours before a weigh-in for a wrestling match for which they lost weight, and 5 to 6 weeks after the end of the wrestling season. There was a mean body weight loss of 4.8 kg prior to the match, of which 3.5 kg was fat-free mass.

To determine if diet composition would affect changes in body composition during energy restriction, Walberg et al. (57) measured body composition and nitrogen balance in 14 male body builders consuming 18 kcal/day for a 7 day period. One group consumed a moderate protein, high carbohydrate diet (MP/HC) which contained the RDA for protein, while the other consumed a high protein, moderate carbohydrate (HP/MC) diet. Weight loss was not significantly different between groups, however MP/HC lost an average of 3.0 kg, while HP/MC lost an average of 2.2 kg. There were no differences observed among groups for percent body fat or fat free mass as measured by hydrostatic weighing, although there was a significant decrease in fat free mass in both groups. Body composition was affected as measured by nitrogen balance. The MP/HC group was in

negative nitrogen balance throughout energy restriction, while the HP/MC group was in positive nitrogen balance. It was suggested that the 2% error associated with hydrostatic weighing might have made this method insensitive to body composition changes during a short period of time. This study suggests that consuming the RDA for protein during energy restriction is not sufficient to counteract the catabolism of body proteins induced by negative energy balance. Towards the end of the 7 day period, the MP/HC group was nearly in nitrogen balance. Because of the body's adaptation to energy restriction over time, repeated, short-term energy restriction of several days may affect fat free mass more than chronic energy restriction.

Losses in lean body mass may indicate losses in muscle protein, since amino acids are used for gluconeogenesis to produce glucose during times of low carbohydrate intake. These losses in lean body mass may correspond with losses in performance. Athletes would clearly prefer to lose body fat and minimize lean tissue loss during weight loss. As a result, dietary and exercise strategies that can prevent lean tissue loss during energy restriction are desirable.

Energy Restriction and Anaerobic Exercise Performance

Athletes in several sports attempt to maximize percentage of lean body mass by energy restriction to lose fat mass, in anticipation of improved exercise performance or attaining a weight class. Severe energy restriction in preparation for competition has been widely reported in wrestlers, resistance trainers and bodybuilders. One drawback to such an approach to controlling body composition is that while fat mass is decreased during energy restriction, muscle mass is decreased as well, and performance can be negatively affected.

There is some research that shows that energy restriction may affect muscle function and metabolism. Walberg et al. (57) measured quadriceps and biceps isometric endurance in 14 male body builders consuming 18 kcal/day for a 7 day period. Repeated isometric quadriceps endurance was significantly reduced in subjects consuming high protein and moderate carbohydrate during energy restriction, and tended to be reduced in those consuming moderate protein and high carbohydrate during energy restriction.

Biceps endurance was unaffected by energy restriction or macronutrient content of the diet.

Walberg-Rankin et al. (59) measured performance in 12 collegiate wrestlers before energy restriction, after energy restriction of 3 days, and after a refeeding period of 5 hours. Total work production and average work performed were measured during 8 maximal bouts of 15 seconds of arm ergometry at 0.04 kg per kg body weight interspersed with 20 seconds unloaded cranking. Total work was reduced by an average of 7.6% after the energy restriction period.

Walberg-Rankin et al. (58) measured peak torque and endurance during elbow flexion and knee extension in 14 weight trainers that were energy restricted 10 days. The mean reduction in peak torque was about 8% during the elbow flexion and knee extension tests after energy restriction. There was an increase in biceps endurance after energy restriction. It was suggested that the increase in endurance may have been the result of decreases in peak torque, since the initial force produced was lower and may have been easier to maintain.

Houston et al. (28) measured muscle force development in wrestlers after 4 days of energy restriction. Peak torque production in the quadriceps was significantly reduced at 30º per second 48 hours and 80 hours after energy restriction began. At 180º and 300º per second, peak torque production was reduced during energy restriction, but the reduction was not significant. The low number of subjects probably contributed to the lack of significant results.

Jeejeebhoy (32) reported that fasted or hypocalorically fed individuals showed impaired muscle function compared to normally fed control subjects. Muscle force production in malnourished individuals was decreased during high-intensity (50 Hz) electrical stimulation. At a lower intensity of stimulation (10 Hz), muscle force production was maintained. The force produced at 10 Hz was significantly closer to maximum in fasted or energy restricted individuals when compared to control subjects. The muscle relaxation rate was significantly slower with fasting (7.6% force loss per 10 milliseconds) compared to control subjects (9.6% force loss per 10 milliseconds). Muscle

fatiguability in malnourished individuals was 13.7% force loss per 30 seconds, which was significantly greater than fatiguability controls (3.5% force loss per 30 seconds).

While the above studies demonstrate that energy restriction may impair exercise performance, there is also evidence that performance can be maintained or improved during energy restriction. Fogelholm (14) reports that studies measuring performance with Wingate tests, vertical jump tests, alactic runs (30 minutes) have found no effect or improvement during energy restriction. In addition, a rehydration period of 5 hours or more can reverse the negative performance effects seen during energy restriction (14).

Several studies show that energy restriction may significantly alter muscle performance. This is an important consideration for athletes considering energy restriction as a strategy to alter body composition by reducing body weight or body fat. Although certain advantages such as aesthetics, a lower weight class, or decreased energy cost may be attained with a decrease in body weight or body fat, they may be attained at a cost of diminished performance due to changes in muscle function.

Energy Restriction and Muscle Creatine

The effect of energy restriction on muscle Cr and CrP is a research focus important to individuals trying to achieve weight loss while maintaining physical performance. This type of research has been aimed at determining what effects energy restriction and subsequent changes in muscle kinetics have on muscle function and the overall function of the individual.

Muscle CrP was decreased in rats and obese individuals during periods of energy restriction (32). In humans on a hypoenergy diet of 400 kcal/day for 2 weeks, and in rats fasted to 25% of their body weight, muscle biopsies showed decreased CrP/ATP ratios. Similar results were found using NMR to measure muscle metabolites. After a 2 day fast, rats showed significantly decreased muscle CrP levels compared to controls as measured by NMR (40). Rats fed hypocaloric diets until body weight was decreased 20% showed CrP levels significantly decreased relative to those on the 2 day fast. It was noted that since ATP levels remained unchanged, the change in ratio was the result of muscle CrP losses.

The author hypothesized that the changes in muscle CrP stores due to energy restriction were secondary to losses in muscle glycogen and enzymes such as phosphofructokinase (32). It was suggested that as a result of decreased glycolytic function, less energy is available for muscle contraction. CrP would then take on a greater role in energy production during high-intensity muscle contraction, and therefore become depleted. It was further noted that the effects of malnutrition on muscle function are not limited to losses in lean body mass. Some changes, such as decreased force and slow relaxation rate are similar to changes in fatigued muscle. Further investigation is necessary to determine the mechanism behind these changes.

Houston et al. (28) measured changes in muscle fuel stores after 4 days of energy restriction in 4 collegiate wrestlers. After a baseline mean of 62.3 mmol/kg, muscle glycogen was significantly reduced 2 days (44.0 mmol/kg) and 4 days (33.9 mmol/kg) following weight loss. Muscle ATP and CrP were unaffected. In addition to a lack of data, the results of Houston et al. (28) and Pichard et al. (40) conflict regarding the effect of energy restriction on muscle CrP. Therefore, further study is warranted.

It appears that energy restriction may result in decreases in muscle CrP stores, and may alter CrP metabolism and ATP production. Since individuals with low initial muscle Cr levels benefit most from Cr supplementation, an athlete with lowered muscle Cr stores due to energy restriction may benefit from Cr supplementation to elevate and maintain these stores above normal levels.

Creatine Supplementation and Exercise Performance

Muscle Phosphocreatine Resynthesis

Greenhaff et al. (19) measured the resynthesis of CrP before and after Cr supplementation in order to study the mechanism of the effect of increased muscle Cr stores on performance. Muscle biopsies were taken from eight male subjects 0, 20, 60, and 120 seconds after twenty 1.6 second electrically evoked isometric quadriceps muscle contractions separated by 1.6 seconds. This protocol had been shown to result in almost total degradation of muscle CrP stores. Ten days later this protocol was repeated after subjects had supplemented with 20 g Cr per day for 5 days. The five subjects that began

supplementation with the lowest initial muscle Cr levels had the largest increases in total muscle Cr, and had an accelerated rate of CrP resynthesis after 40 seconds of recovery. The subjects that did not have an increase in muscle Cr due to supplementation did not have an increase in CrP resynthesis. There was no difference in CrP resynthesis during the first 40 seconds of recovery. The authors concluded that some individuals are responders to Cr supplementation while others are nonresponders. Also, it was suggested that the increase in CrP resynthesis after 40 seconds was due to the effect of increased muscle Cr levels on the CPK reaction as free Cr levels approached the Km for free Cr. This study showed that performance improvements after Cr supplementation may be the result of an increase in CrP resynthesis during recovery.

Studies That Show No Effect

Currently, the literature is divided as to the benefits of Cr supplementation. Research concerning Cr supplementation has not always resulted in performance improvements. Burke et al. (7) studied the effect of supplementation of 20 g of Cr per day for 5 days on a single-effort sprint in 32 elite swimmers. The swimmers performed sprints of 25, 50 and 100 meters with 10 minutes of active recovery between each test as well as a 10 second leg ergometry test. No significant differences were found between the control group and the experimental Cr loaded group. It was concluded that Cr supplementation does not enhance single-effort performance in elite swimmers.

Redondo et al. (41) tested the effects of supplementation of 20 g of Cr per day for 7 days on sprinting performance in a 60 meter sprint. Eighteen well trained athletes performed three 60 meter sprints with 2 minutes of rest before and after seven days of Cr supplementation. No significant differences were found following supplementation that would indicate an ergogenic effect of Cr supplementation on sprint running performance.

Cooke et al. (8) studied whether Cr supplementation would affect power output and fatigue during bicycle sprinting. Twelve healthy but untrained male subjects performed 2 cycling sprints separated by 20 minutes of rest to determine power output and fatigue before and after supplementation of 20 g of Cr per day for 5 days. No

significant differences were observed between placebo and supplemented groups before or after supplementation.

Mujika et al. (36) measured sprint performance in 20 highly trained swimmers before and after Cr supplementation. Sprints of 25, 50, and 100 meters in the swimmers' best strokes were performed under competition-like conditions and 20 to 25 minutes of passive recovery were allowed between sprints. Subjects supplementing with Cr tended to perform worse after supplementation, but there were no significant differences between groups.

Thompson et al. (51) studied calf muscle metabolism and 100 meter and 400 meter swim performance before and after 6 weeks of supplementation with Cr placebo at 2 g/day. Swim times and calf exercise duration did not change after Cr supplementation. There was no reported subjective benefit of supplementation.

Terrillion et al. (50) investigated whether Cr supplementation of 20 g/day had an effect on running performance during two 700 meter runs. Trials were separated by 60 minutes, and were performed before and after supplementation with Cr or placebo. There was no effect of Cr supplementation on running time.

Snow et al. (46) measured performance in a single sprint cycling effort of 20 seconds after Cr supplementation. Eight active but untrained men supplemented for 5 days with 25 g of creatine or dextrose per day. Performance in the first 20 second cycle sprint was measured 4 weeks prior to the second cycle sprint, and supplementation occurred 5 days prior to the second cycle sprint. Peak power, mean power, time to peak power, and percent power decrement were not affected by Cr supplementation. It was concluded that Cr supplementation does not result in a change in performance during single-effort exercise.

It is important to note that the only studies mentioned above that measured muscle Cr levels were that of Thompson et al. (51) and Snow et al. (46). The subjects in Thompson et al. (51) had no change in muscle Cr following supplementation. It is possible that the lack of significant performance improvements reported by Thompson et al. (51) and others was the result of some subjects being nonresponders. The Cr supplemented subjects studied by Redondo et al. (41), Thompson et al. (51), and

Terrillion et al. (50) did not demonstrate an increase in body mass, indicating that the Cr supplementation protocol may not have raised Cr levels. It has been suggested that elite or well trained athletes may benefit less from Cr supplementation than untrained athletes, because their muscle Cr stores may already be maximized (37). This may have been the case in the studies by Burke et al. (7) Redondo et al. (41), Thompson et al. (51) and Terrillion et al (50).

CrP resynthesis after depletion from high-intensity exercise occurs in two phases (56). The fast phase has a half-time of about 22 seconds, while the half-time of the slow phase is greater than 170 seconds. Therefore, CrP is almost fully resynthesized after 4 minutes of rest. Repeated exercise bouts of high intensity and short rest may be more likely to be affected by improved Cr status than single bouts of exercise. The studies by Burke et al (7), Cooke et al. (8), Mujika et al. (36), and Snow et al. (46) involved single effort performances, or repeated bouts with rest of 10 minutes or longer. Cr supplementation does not appear to benefit exercise performance of this type.

Studies on Endurance Performance

Balsom et al. (3) measured performance during a supramaximal treadmill run and a ~6 kilometer terrain run before and after Cr supplementation. Performance in the treadmill run was not different between the Cr supplemented group and the placebo group. During the terrain run, there was no change in performance for the placebo group, while the Cr group had a significant increase in run time. While there was no relationship between increases in body mass in the Cr group and increases in terrain run times, it was suggested that body mass increases as a result of Cr supplementation were the cause of performance impairment.

Engelhardt et al. (11) measured cycle performance in 12 competitive triathletes before and after low-dose Cr supplementation. All subjects consumed 12 g of Cr per day. After a 30 minute endurance ride, 4 periods of interval exercise were performed consisting of ten 15 second sprints at 7.5 W/kg with 45 seconds rest. Interval exercise was stopped if the subject could not maintain intensity. After the 4 interval periods, the 30 minute endurance ride was repeated. Six subjects could not complete the second endurance ride

during the first trial, and while they improved the length of their endurance ride after Cr supplementation, this improvement was not significant. Following Cr supplementation, the number of repetitions performed during interval periods 1 and 2 was significantly increased. It was noted that 75% of the subjects improved performance after supplementation, and no subjects exhibited a drop in performance. It was concluded that Cr supplementation at a low dose has positive effects on sprint performance within aerobic exercise.

Rossiter et al. (42) investigated the effect of Cr supplementation for 5 days at 0.25 g per kg on 1,000 m rowing performance in competitive male and female rowers. Rowing times decreased in subjects supplementing with Cr, while those of subjects ingesting placebo did not change. Further, performance enhancement was greatest during the final 200 m sections. It was concluded that Cr supplementation was effective in enhancing performance during 1,000 m rowing trials.

Febbraio et al. (13) assessed the effect of Cr supplementation on metabolism and performance during four 1 minute cycling sprints separated by 1 minute rest, followed by a fifth bout to fatigue. Performance tests were performed after 5 days of Cr supplementation and after 28 days without supplementation. There were no differences in exercise duration during the fifth bout. Muscle Cr and CrP were significantly elevated in Cr supplemented subjects as measured in muscle biopsy samples. It was concluded that increases in muscle Cr as a result of supplementation do not affect performance during exercise in which CrP is not the principal source of energy.

The available literature demonstrates that Cr supplementation may have a beneficial effect on certain types of endurance performance. This ergogenic effect depends on the type of exercise, and appears to be most likely when the exercise involves high-intensity exercise interspersed with endurance exercise. This type of exercise may be more clearly defined as maximal endurance, or the length of time that maximal exercise can be maintained. This has been shown in both single and multiple bouts of exercise (11,42). In high-intensity exercise to exhaustion, performance was not affected by Cr supplementation, and performance in traditional endurance exercise was impaired. (3,13).

Studies Showing Swimming Performance Enhancement

Grindstaff et al. (22) measured repeated sprint swim performance in 18 male and female competitive swimmers before and after 9 days of supplementation with Cr or placebo. Sprint tests included three 100 m freestyle swims with 60 seconds of rest between each, as well as three 20 second arm ergometer sprints with 60 seconds rest. Creatine supplemented subjects swam significantly faster in the second and third 100 meter sprint swims than the placebo group, and also improved their swim times relative to pre-supplementation. Improvements in work and power during sprint 1 of arm ergometry were also greater in the Cr group.

S*tudies Showing Weight Lifting Performance Enhancement*

Earnest et al. (10) measured weight lifting performance in 8 strength trained athletes before and after Cr supplementation of 20 g of Cr or placebo per day for 28 days. Strength was measured as the maximum weight a subject could successfully lift 1 time in any lift (1 RM). Bench press 1 RM was significantly increased in the Cr group. When expressed relative to body weight, no differences were found between groups, as the Cr group had a significant increase in body weight. Total lifting volume was significantly increased in the Cr group in both absolute and relative terms.

Kreider et al. (34) studied 25 NCAA division IA football players to determine if supplementation of Phosphagen HP (Experimental and Applied Sciences, Golden, CO) or Phosphagen HP plus Cr would improve bench press, squat, and power clean performance. Subjects were resistance and agility training for 8 hours per week throughout the study. The daily intake of Phosphagen HP contained 99 g of glucose, 3 g taurine, 1.1 g disodium phosphate, and 1.2 g potassium phosphate, and the Cr group consumed an additional 15.75 g/day of Cr. Improvements in bench press lifting volume and sum of bench press, squat, and power clean lifting volume were greater in the group supplementing with Cr. It is important to note that the strength tests used were based on observations made by strength coaches working with the athletes, in order to mimic the athletes' training. The weight used for each athlete was determined by estimating a weight at which the athlete could complete a maximum of 4 to 8 repetitions. Lifting volume was defined as the

number of repetitions multiplied by the amount of weight lifted. The authors concluded that adding Cr to the glucose/taurine/electrolyte supplement allowed for greater improvements in lifting volume than the supplement alone.

Vandenberghe et al. (54) measured arm-flexion torque and muscle strength in 19 sedentary females before and after Cr supplementation. Supplementation began with 4 days of ingesting 20 g of Cr or placebo per day, and subsequently continued for a 10 week period where supplementation was 5 g/day. For 1 hour, 3 times per week, subjects underwent resistance training involving seven different basic lifts. A 10 week detraining period followed supplementation, in which training was terminated but supplementation continued. Strength was defined as the 1 RM for the various lifts, and arm-flexion torque was measured with an isokinetic dynamometer. Strength increases after the 10 week training period were significantly greater for the Cr supplemented group than the placebo group in leg press, leg extension, and squat. Maximal intermittent exercise capacity of the arm-flexors was significantly increased. These measures remained elevated in Cr supplemented subjects until 4 weeks after the cessation of supplementation.

Volek et al. (55) measured performance changes in a bench press and jump squat exercise protocol as a result of ingestion of 25 g of Cr or placebo per day for 6 days. Lifting performance was not altered in subjects consuming placebo. Significant improvements in peak power output were found during 5 sets of 10 jump squats at 30% of 1 RM. Significant increases in repetitions to failure were found during 5 sets of 10 bench presses at 10 RM.

Kelly et al. (33) measured near-maximal muscular strength and high-intensity bench press performance in 18 male powerlifters with > 2 years of experience. Cr supplementation occurred in 2 phases. First, subjects consumed either 20 g of Cr or placebo for 5 days, then for the next 21 days consumed either 5 g of Cr or placebo. Increases in 3 RM strength occurred in both Cr and placebo supplemented subjects, but were greater in subjects ingesting Cr. Significant increases in number of repetitions occurred in the Cr group, but not in the placebo group.

Noonan et al. (38) assessed the effects of different doses of Cr on strength, 40 yard dash time, and vertical jump in 39 male college athletes. Subjects supplemented with 20 g

of Cr or placebo for 5 days, then for 8 weeks consumed either a placebo, 100 mg of Cr per kg of fat free mass, or 300 mg of Cr per kg of fat free mass. Both groups supplementing with Cr significantly improved bench press 1 RM, but only the 300 mg of Cr per kg of fat free mass group had significant improvements relative to the placebo group. Forty yard dash time was significantly improved in subjects supplementing with 100 mg of Cr per kg, but did not change in the other groups. There were no significant differences among groups in vertical jump.

Studies Showing Cycling Performance Enhancement

Balsom et al. (2) investigated cycling performance before and after Cr or glucose supplementation of 25 g/day for 5 days. The exercise protocol progressed in 2 stages. First, 10 bouts of 6 seconds each with 30 seconds rest were performed at 130 revolutions per minute (EX_{130}). The resistance was based on familiarization trials in which the greatest resistance each subject could sustain for 10 bouts was determined. Next, 10 bouts of 6 seconds each with 30 seconds rest were performed at 140 revolutions per minute (EX_{140}). The second stage of the performance test was designed to elicit fatigue, so that 140 revolutions per minute was not sustainable for 10 bouts. After supplementation, the Cr supplemented group was able to perform significantly more work during EX_{140}, while performance of the glucose supplemented group did not change. It was concluded that Cr supplementation could delay the onset of fatigue during repeated bouts of high-intensity cycling.

Birch et al. (6) measured the effects of Cr supplementation on performance in 14 healthy male subjects. Subjects performed 3 maximal isokinetic cycling bouts of 30 seconds with 4 minutes of rest between bouts before and after supplementation of 20 g of Cr or placebo per day for 5 days. Peak power output, mean power output and total work were improved after Cr ingestion in bouts 1 and 2. These measures were unaffected during bout 3.

Earnest et al. (10) measured the performance of 8 strength trained athletes during 3 Wingate bicycle tests with 5 minutes of rest. Following supplementation of 20 g of Cr

or placebo per day for 14 days, 4 subjects significantly increased the total anaerobic work performed, while no changes were found in the placebo group.

Söderlund et al. (47) measured cycling performance in 8 male subjects before and after Cr supplementation of 30 g per day. The cycling protocol consisted of five 6 s bouts with 30 s rest between each followed by a 10 s bout. The resistance was chosen so that a target speed of 140 revolutions per min could be maintained during the 6 s bouts but not for the 10 s bout. Therefore, the work did not change during the 6 s bouts before or after Cr supplementation. After Cr supplementation, all subjects significantly improved their maintenance of the target speed and increased total work performed during the 10 s cycling bout.

Schneider et al. (43) investigated the effect of Cr supplementation on performance during 5 cycling sprints of 15 seconds with 1 minute rest, and 5 cycling sprints of 1 minute with 5 minutes rest. After baseline testing nine subjects supplemented with 30 g of glucose per day for 5 days before the 15 second sprints, then after an additional 2 days of glucose supplementation, the 1 minute sprints were performed. Two weeks later, this protocol was repeated using 25 g of Cr plus 5 g of glucose per day as a supplement. Cr supplementation resulted in a significant increase in total work performed during each 15 second bout compared to the placebo group. No differences were found in total work performed during the 1 minute sprints, and Cr supplementation did not result in a decrease in the rate of decline during the repeated bouts.

Studies During Energy Restriction

Only one study has been published that tested the effect of Cr supplementation in energy restricted athletes. Ööpik et al. (39) studied 6 well trained male karate athletes before and after a 5 day period of Cr or placebo supplementation in combination with body mass reduction. Subjects supplemented with 20 g of Cr or placebo in a cross-over design, with weight loss periods separated by 1 month. Subjects were allowed to choose their method of weight loss during both body mass reduction periods. Maximal exercise performance was measured as peak torque and work performed at peak torque during an isokinetic test of the knee extensor muscles at angular velocities of 1.57, 3.14, and 4.71

radians per second. Submaximal endurance was measured as work performed during 45 seconds of knee extensions at a rate 30 contractions per minute, and total work performed. Body mass reduction periods resulted in weight losses of 3 to 4.3% was significantly greater during the placebo trial compared to the Cr trial. There were no significant differences between Cr and placebo groups for work performed at peak torque or peak torque at any angular velocities. However, placebo subjects exhibited decreases in peak torque and work performed at peak torque at 4.71 radians per second as a result of body mass reduction while Cr subjects did not. The amount of submaximal work was significantly lower in Cr subjects after supplementation. The placebo group also exhibited decreases in submaximal work performed, but the decrease was not significant.

One drawback to this study is the allowance that subjects could choose their method of body mass reduction, although the method used was kept similar for both weight loss periods. Therefore, it is not known what effects different weight loss methods such as energy restriction, dehydration, and increased energy output may have had on Cr supplementation, since subjects used different approaches to weight loss. While the subjects were asked to reduce their body mass by 5% over 5 days, the subjects lost between 3 and 4.3 % of body weight on average.

Summary

It has been repeatedly shown that supplementation with Cr can benefit exercise performance in high-intensity, intermittent exercise. Several studies found strength improvements in 1 maximal repetition (10,38,54), although other types of single effort exercise have not shown improvements with Cr administration (7,8,37,46). The only study that has measured performance changes during body mass reduction found an ergogenic effect of supplementation on peak torque and work performed during peak torque, but found a performance decrement during submaximal work. The ergogenic benefit of Cr supplementation appears to be related to an increase in Cr storage within the muscle, and a subsequent increased rate of CrP resynthesis during recovery between repeated bouts of exercise (18,19).

Needle Muscle Biopsy and Muscle Creatine Degradation

There is some debate in the literature about the use of the needle muscle biopsy technique in obtaining measurements of muscle Cr and CrP. Nuclear magnetic resonance studies in general report higher CrP concentrations than biopsy studies, and it has been suggested that the biopsy procedure may allow degradation of CrP in the sample prior to and during freezing (49). However, it has also been suggested that NMR may overestimate CrP concentrations as a result of difficulties with calibration and measurement. Essen et al. (12) found that a 1 minute delay in muscle freezing after biopsy had no effect on resting CrP concentrations. Söderlund and Hultman (48) studied the effects of delayed sample freezing as well as the freezing process itself. Muscle CrP increased 16% in the minute following biopsy, but delays up to 6 minutes had no further effect. Freezing was also found to have no effect on CrP concentration. Based on the small amount of evidence regarding the effects of measurement technique on muscle creatine contents, it appears that the needle muscle biopsy technique may result in only small changes in CrP concentration.

CHAPTER III JOURNAL MANUSCRIPT

THE EFFECT OF CREATINE SUPPLEMENTATION ON MUSCLE FUEL STORES, BODY COMPOSITION, AND EXERCISE PERFORMANCE DURING ENERGY RESTRICTION

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ABSTRACT

The purpose of this investigation was to determine the effects of a four day creatine load and simultaneous energy restriction on muscle creatine content, exercise performance, and body composition in 24 male recreational resistance trainers, age 18-26. Sixteen subjects were randomly divided into placebo (Pl, n=8) and creatine supplement (CrS, n=8) groups. Control (C, n=8) subjects of the same age were recruited separately to complete the performance and body composition tests while consuming their normal diet. The CrS group was administered 20 $g \cdot d^{-1}$ of creatine monohydrate (Cr) mixed with 5 g/d of sucrose, while the Pl group was administered 25 $g \cdot d^{-1}$ of sucrose. Both CrS and Pl consumed a formula diet of 75.3 kJ (18 kcal) $\cdot kg^{-1} \cdot d^{-1}$ for 4 d. Testing before and after energy restriction consisted of a repeated sprint cycle performance test (10 sprints of 6s, with 30s rest), hydrostatic weighing, and resting needle muscle biopsy. Testing revealed that subjects in CrS and Pl demonstrated significant decreases in body weight and % body fat (%BF) with no difference between groups. However, Pl demonstrated a significantly greater % loss in FFM (2.4 ± 0.25%) compared to CrS (1.4 ± 0.4%) ($p<0.05$). The muscle fuel stores of CrS and Pl responded significantly to the diet. Significant increases in muscle total Cr ($p<0.01$), free Cr ($p<0.01$), and CrP ($p<0.05$) of 16.5%, 16.8%, and 16% respectively were demonstrated by CrS over the energy restriction period, while Pl demonstrated significant decreases of 7.2% and 8.2% respectively in muscle total Cr ($p<0.01$) and free Cr ($p<0.05$). There were no significant differences between groups for performance during the cycle test, however, there were trends towards performance enhancement in CrS, as total work ($p=0.078$) and work capacity ($p=0.058$) increased 3.8 ± 2.2% in CrS and decreased 0.5 ± 0.4% in Pl. It was concluded that short-term energy restriction resulted in decreased muscle Cr storage, and that Cr supplementation during energy restriction increased muscle Cr and CrP stores. Consumption of Cr caused CrS to lose a significantly lower %FFM compared to Pl. Cr supplementation resulted in trends toward improved performance in CrS relative to Pl after energy restriction, but did not influence losses in body weight or %BF.

Introduction

Athletes often restrict energy intake as part of training or in preparation for competition, with a goal of reducing body weight. Reductions in body weight associated with energy restriction consist of body fat, which is desired, and lean body mass which is not (17,23). Losses in body fat are expected to aid performance due to decreased energy cost of exercise. Losses in lean body mass are undesirable because muscle loss may diminish performance.

Several studies have determined that energy restriction results in decrements in anaerobic exercise performance in athletes, even when the goal of reduced body weight is attained (17,18,23,36,37,38). Walberg-Rankin et al. (37) reported that peak torque during elbow flexion and knee extension was significantly reduced in male weight trainers after 10 d of energy restriction. Houston et al. (18) found that peak torque production in the quadriceps was significantly reduced at $30^{o} \cdot s^{-1}$ after energy restriction and dehydration. Further, at $180^{o} \cdot s^{-1}$ and $300^{o} \cdot s^{-1}$, peak torque was significantly reduced when expressed as a percentage of the initial values. Energy restriction has also been reported to result in improvements in some indicators of muscle function, such as biceps endurance and vertical jump, and to cause no impairment to others (9,37). While energy restriction may improve performance in some ways, it is likely to diminish performance in others.

Energy restriction may have an effect on muscle Cr and CrP stores. In rats and obese humans on severe energy restriction, muscle CrP stores have been shown to be reduced (26). It was suggested that the reductions in muscle CrP were secondary to losses in muscle glycolytic and oxidative enzyme activity, which resulted in an increased contribution of the phosphagen system to energy production. Houston et al. (18) found that CrP levels were unchanged in wrestlers during energy restriction with dehydration. It has not been determined whether energy restriction of the magnitude and practiced by athletes preparing for a competition will result in changes in muscle Cr. The only study to our knowledge that has measured the effects of Cr supplementation during energy restriction did not measure muscle Cr content (25). However, performance during

isokinetic knee extensions was maintained in the Cr supplemented subjects after weight loss and supplementation, while performance diminished in the placebo group.

A strategy for performance enhancement that has received a great deal of attention recently is the supplementation of creatine monohydrate (Cr). Harris et al. (16) found that a supplementation regimen of consuming 5 g of Cr, 4 times per day for a period of 4 to 6 days resulted in muscle Cr elevations of about 20%, and that both Cr and CrP contributed to the increase in total Cr composition. This quantity is vastly greater than the 2 g found in the average daily diet (12). Further, an increase in muscle Cr content has been associated with performance improvements during high-intensity, intermittent exercise (1,3,6,7,21,22,28,29,33,35). The mechanism for this performance enhancement has been shown to be associated with an increase in the resynthesis rate of CrP during recovery, as a result of the effects of increased free Cr concentrations on the creatine phosphokinase (CPK) reaction (13). Other studies have found no effect of Cr supplementation on performance, especially in single-effort or endurance exercise (2,4,5,8,24,27,29,31,32). Creatine supplementation has been found to result in increases in body mass and FFM, which may be due to increased total body water retention, muscle fiber size, and protein synthesis (6,19,22,35).

Energy restriction has been shown to result in losses in FFM and muscle CrP, and diminished performance, while Cr supplementation has been shown to result in increases in lean body mass and muscle Cr, and enhanced performance. Therefore, the purpose of this study was to determine whether Cr supplementation during energy restriction counteracts the negative effects associated with body weight reduction in athletes.

Methods

Subjects. Sixteen actively training male resistance trainers age 18 to 26 gave their informed consent and volunteered to participate in this study, which was approved by the Institutional Review Board. Subjects had trained for at least 2 years, 3 to 6 times per week, with no reported history of anabolic steroid use. Eight of the subjects had supplemented with Cr previously, but none had supplemented for at least 1 month prior to the study. Subjects were screened for contraindications to weight loss and resistance

exercise such as diabetes, heart conditions, orthopedic limitations and injuries, and major organ malfunctions. Subjects were randomly assigned to 2 groups: creatine supplementation (CrS), and placebo (Pl). Eight control (C) subjects of the same age were recruited separately.

Protocol. Body weight maintenance prior to the start of energy restriction was determined by weighing subjects 2 weeks prior to the intervention. The maximum allowable body weight change over the 2 weeks was 1.0 kg. Practice performance tests (Tests 1 and 2) were performed 2 d and 4 d prior to the intervention.

On the first morning of the intervention, subjects reported to the laboratory between 7:00 and 9:00 a.m. after a 12 hour fast for baseline performance and body composition testing. At 1:00p.m., subjects reported to the training room where muscle biopsies were taken. Subjects began the formula diet after baseline performance and body composition testing in the morning of day 1. Each subject was given 450 kcal of food to consume between baseline testing and the first biopsy. After the biopsy, subjects received two supplement packets, and their remaining food for the day. The hypoenergy diet of 18 kcal $\cdot$ $kg^{-1}\cdot$ d^{-1} began immediately after the biopsy. Subjects reported to the laboratory each day of the intervention for body weight assessment. Body weight was assessed to the nearest 0.1 kg on a medical scale each morning of the experimental period after an overnight fast. After weighing each morning, subjects were given food and four supplements for the day. On the fifth morning of the intervention, performance and body composition testing was repeated, at the same time as they had been performed on day 1. After completion of post-intervention performance and body composition testing, each subject was given 450 kcal of food to consume prior to the muscle biopsy that afternoon. The formula energy restriction diet ended after completion of the second muscle biopsy.

The diet consisted entirely of Ensure High Protein, Ross Laboratories, with the proportion of total energy as 54.7% carbohydrate, 21.3% protein, and 24.0% fat. The subjects consumed no other Calories, although water and non-caloric drinks were consumed ad-libitum. The control group ate an unrestricted diet throughout the experiment, and did not undergo muscle biopsy. Placebo subjects consumed 6 g of sucrose four times per day, while CrS consumed 5 g of Cr plus 1 g of sucrose, four times

per day throughout the energy restriction period. Subjects were instructed to mix the powdered supplement into a can of Ensure just prior to consumption at morning, mid-day, afternoon, and evening, since it has been shown that Cr accumulation is greater when consumed with carbohydrate (11). Subjects maintained their regular exercise routine by weight lifting two to three times throughout the intervention.

Performance Test. The repeated sprint performance test, a modification of that shown by Gaitanos et al. (10) to cause a 84% reduction in muscle CrP after the final sprint, consisted of 10 repeated 6 s leg sprints separated by 30 seconds of passive recovery on a modified Monark cycle ergometer. The ergometer was integrated with a microcomputer and software which recorded the following as described by Williams et al. (39): peak power (W, the highest power output for ½ pedal stroke), total work (kJ, the total amount of work performed during all ten sprints), and fatigue index (%, the difference in peak power or total work between sprint 1 and 10 in relation to peak power or total work in sprint 1). Work capacity (J/kg) and maximal power (W/kg) were also computed.

Subjects remained seated throughout exercise and recovery. Resistance during the maximal effort cycling bouts was 0.075 kg · kg body weight^{-1}, hung from a strap around the flywheel. Tests 1 and 2 were separated by 24-48 hours and performed between 2 and 4 d prior to the experimental period to reduce the learning effect. The performance tests (Tests 3 and 4) occurred prior to and after energy restriction. Reliability of the performance test was assessed using data from Tests 2 and 3.

Body Composition. Hydrostatic weighing of subjects before and after the experimental period determined the effects of energy restriction and Cr supplementation on body composition. A load cell attached to a chair submerged in a hydrostatic weighing tank (Novel Products, Rockton, IL) sent the information on underwater weight to a computer. The 3 highest weight values obtained from 8 measurements were used to determine percent body fat (%BF) and fat-free mass (FFM). Residual volume of the lungs was measured by the oxygen dilution technique, where subjects breathe five breaths into an oxygen filled rubber bag (39). This procedure was performed twice for each subject, then averaged.

Muscle Samples. Muscle biopsies were taken in the afternoon on day 1 and at the same time of day on day 5. A sample from the lateral portion of the vastus lateralis muscle was removed using the percutaneous needle muscle biopsy technique with suction. A local anesthetic (lidocaine) was injected in several punctures following cleansing. After 4 to 5 minutes, an ~ ½ inch long and deep incision was made in the leg. The needle was inserted into the incision and the sample was removed. The sample was immediately frozen in liquid nitrogen and stored at -80°, freeze-dried, dissected to remove blood, fat and connective tissue, powdered, and stored at -80°. An extract was obtained from the powder using Perchloric Acid and neutralized with $KHCO_3$ (15). The neutralized extract was analyzed for free Cr, CrP, and ATP content using enzymatic spectrophotometric techniques (15).

Statistical Procedures. A two way ANOVA with repeated measures was used to assess differences between groups for the performance tests, muscle biopsies and body composition tests. The Tukey post-hoc procedure was used to discriminate between averages when a significant F-ratio was calculated. One-way ANOVAs were performed on baseline measurements to determine pre-testing differences between groups, and on percent change values to determine differences between groups after the experimental period. Significance was defined as $p<0.05$.

Results

There were no significant differences between groups for age, height, weight, or lifting experience prior to the experimental period. There were no significant differences between subject body weight two weeks prior to the experiment and body weight the first day of the experiment.

Body Weight. There was no change in body weight in any group over the 2 week maintenance period. There was a significant time effect of the experiment, as body weights in both Pl and CrS dropped significantly over the course of the experiment (Fig. 1). However, there were no differences between groups any day of the experiment. There was also no difference in the % change in body weight. Mean body weight loss for CrS

was 2.2 ± 0.4 kg (2.7%), while mean loss for Pl was 2.75 ± 0.2 kg (3.6%). The mean body weight gain in C was 0.05 ± 0.15 kg (0.07%).

Performance. The reliability of the performance test was assessed by correlating data from tests 2 and 3, and by analyzing performance in C. The correlation for total work performed over all 10 sprints was r=0.89, and for peak power was r=0.86. The correlation between tests 3 and 4 for C for total work, peak power, total work fatigue ratio, and peak power fatigue ratio was r=0.99, r=0.99, r=0.85, and r=0.90 respectively.

There were no significant differences or interactions between groups before or after the energy restriction and supplementation period for total work, work fatigue, work capacity, peak power, peak power fatigue, or maximal power. There were also no significant differences as measured by % change in performance from pre- to post-energy restriction. There was a group by time interaction for work performed during bout 4 ($p<0.05$), as CrS demonstrated a mean increase in work after the experimental period of 4.6 ± 2.1%, while Pl demonstrated a mean decrease of 1.1 ± 1.0%. It is important to mention that this can not be considered significant because of the family-wise error rate of $\alpha<0.005$. There were no significant performance differences in C in any measure, pre- or post-experiment.

There was a trend toward a group by time interaction for performance as measured by total work (Fig. 2) ($p=0.078$) and work capacity (Fig. 3) ($p=0.058$). Trends were also indicated by % change for total work ($p=0.072$) and work capacity ($p=0.072$), as both measures increased 3.8 ± 2.2% in CrS and decreased 0.5 ± 0.4% in Pl. When the data were analyzed for individual exercise bouts, there were trends toward group by time interactions at sprints 2 ($p=0.086$), 7 ($p=0.098$), and 10 ($p=0.091$). Also, CrS demonstrated trends toward increased work performed as a group during bouts 3 ($p=0.067$) and 4 ($p=0.086$). Although there appear to be indications of performance enhancement during these individual bouts at the $p<0.1$ level, these can not be considered significant when compared to the family-wise error rate of $\alpha<0.005$.

There was a significant increase in maximal power but no interaction between groups over time on this factor (5.7 ± 2.7% increase in CrS and 1.7 ± 1.7% increase in Pl, $p<0.05$) (Fig. 4). While peak power fatigue decreased 10.0% in CrS, and increased 3.2% in Pl, and peak power increased 2.3% in CrS and decreased 2.0% in Pl, these interactions

were not significant. The performance data of one subject in CrS were dropped from all performance analysis because a toe clip broke during test 4.

Muscle Metabolites. Prior to the experimental period, Pl had significantly greater total Cr stores relative to CrS (Table 2). There was a significant group by time interaction for total Cr, since energy restriction and supplementation caused a significant mean increase in muscle total Cr of 16.5% in CrS, and a significant mean decrease in muscle total Cr of 7.2% in Pl (Fig. 5). Similarly, muscle free Cr stores were significantly greater in Pl than CrS prior to the experiment and there was a significant group by time interaction. The Cr supplemented group demonstrated a significant mean increase in muscle free Cr of 16.8% while the Pl group demonstrated a significant mean decrease in muscle free Cr of 8.2%. There were no significant pre-experiment differences between groups for muscle CrP. There was a significant group by time interaction, as there was a significant increase of 16% in the CrP stores of CrS and a non-significant mean decrease of 6% in Pl. Muscle ATP was not affected by energy restriction or supplementation. The muscle metabolite data of 2 subjects in CrS and 2 subjects in Pl were dropped from the analysis due to unusable muscle biopsy samples.

Body Composition. There were no significant pre-experiment differences between groups for %BF or FFM (Table 3). There was a significant time effect on %BF, as it significantly decreased 8.8 ± 1.9% and 6.1 ± 1.7% in CrS and Pl respectively over the experimental period. Oneway ANOVA revealed a significant difference between groups when FFM was expressed in terms of % change, as FFM decreased 1.4 ± 0.4% in CrS and 2.4 ± 0.25% in Pl. Percent change in %BF was not different between groups. Post-experimental %BF and FFM did not differ between Pl and CrS. Percent body fat and FFM did not change in C.

Discussion

Muscle Cr and CrP stores. Recreational resistance trainers that supplemented with Cr during energy restriction demonstrated significant increases in muscle Cr and CrP while those that supplemented with a placebo during energy restriction demonstrated significant decreases in muscle Cr. The pre-experiment difference between Pl and CrS in our study

may have contributed to the robust effect on muscle fuel stores, as those individuals with initial total Cr levels below 120 mmol · kg^{-1} dm generally show the greatest increases with supplementation (13,16). Similar to Harris et al. (16), we found that the increase in muscle total Cr was the result of increases in both free Cr and CrP.

Previous studies using rats and obese subjects have shown that energy restriction may result in muscle CrP losses, although this has not been shown in athletes (18,20). Our data agree with Houston et al. (18), however, in revealing no significant muscle CrP change in Pl after energy restriction. Unfortunately, they did not measure changes in free Cr or total Cr, which is where we found the most dramatic changes in muscle Cr storage.

Pichard (26) measured muscle CrP and total Cr by P^{31} NMR spectroscopy in rats on a 2 d fast, and rats chronically fed a hypocaloric diet until they lost 20% of their body weight. The CrP stores of rats on the 2 d fast and the 20% hypocaloric diet were 30% ($p<0.01$) and 45% ($p<0.01$) lower than control rats, respectively. Our Pl subjects demonstrated a loss in CrP, however the mean 6.25% drop in muscle CrP was not significant. The change in CrP may not have been as dramatic due to the lack of severity of the energy restriction in our study relative to that of Pichard et al. (26).

Our use of a creatine-free formula diet may also have contributed to the decreases in muscle free and total Cr in Pl. Harris et al. (16) reported that endogenous synthesis of Cr was sufficient to maintain muscle Cr levels, since two subjects in the study were vegetarians with muscle Cr levels within the normal range. Greenhaff (12) reported that endogenous synthesis of Cr is depressed when Cr intake is high, but returns when Cr intake is low. In agreement, Pichard et al. (26) only found significant changes in muscle total Cr in acutely fasted rats. Our study demonstrated the same effect in resistance-trainers. It is possible that the decreases seen in our study were a result of the short-term consumption of a Cr-free diet, and that the free Cr levels, and therefore total Cr levels of Pl would have returned to baseline in time as endogenous Cr synthesis increased. Rats chronically fed a hypocaloric diet by Pichard et al. (26) demonstrated a slight, but insignificant increase in total Cr. Therefore, it appears that the decrease in muscle total Cr seen during acute energy restriction may be reversed in chronically energy restricted individuals, and may be related to both energy and Cr content of the diet.

Harris et al. (16) reported mean total Cr levels of 126.8 ± SD11.7 mmol · kg^{-1} dry muscle (dm) in seventeen subjects prior to supplementation, while the sixteen subjects in our study demonstrated pre-experiment mean total Cr levels of 121.6 ± SD7.6 mmol · kg^{-1} dm. The mean 14.1% increase in muscle total Cr in CrS was slightly lower than that reported by Harris et al. (16) using subjects in energy balance, since they all demonstrated post-supplementation total Cr levels greater than 140 mmol · kg^{-1} dm, and six subjects demonstrated levels over 150 mmol · kg^{-1} dm. Although CrS demonstrated an increase in muscle Cr storage, it appears that energy restriction may have reduced the uptake of Cr into the muscle. Since Green et al. (11) demonstrated that carbohydrate ingestion augments uptake of Cr, it is possible that the reduction in carbohydrate and food intake during energy restriction affects muscle Cr accumulation. Ööpik et al. (25) suggested that Cr uptake in their subjects may have been impaired, as evidenced by plasma ammonia changes during exercise. Supplementation was expected to improve ATP homeostasis in muscle, and therefore affect ammonia metabolism, since supplementation in energy balance has been shown to result in a reduction in ammonia in the blood after exercise (14). However, the changes in ammonia metabolism found by Ööpik et al. (25) were tendencies and did not reach significance, suggesting that Cr supplementation did not have as great an effect on ATP homeostasis during weight loss as is seen during energy balance.

Dietary supplementation of Cr has been shown to significantly elevate muscle Cr and CrP storage in energy balance (16). Our study is the first, to our knowledge, to show that a similar effect is seen during energy restriction. Ööpik et al. (25) found that subjects lost more body weight when supplementing with placebo than with Cr during energy restriction, suggesting changes in muscle Cr stores. However, they did not measure these stores directly. Our study suggests that Cr supplementation can counteract the drop in muscle Cr levels seen with acute energy restriction, and result in an increase in muscle Cr and CrP, although these elevations may be lesser than those seen with supplementation during energy balance.

Performance. Previous work from this laboratory and others has shown that energy restriction and the rapid loss of body mass can result in impairments in high-intensity exercise performance (18,36,37,38), although it has also been shown to cause

improvements in performance measures such as biceps endurance and vertical jump (9,37). Our study showed that high-intensity intermittent cycling performance was not significantly altered in Pl as a result of energy restriction. However, Pl did not exhibit significant changes in muscle CrP, the major energy source for this type of exercise. As a result, the lack of an effect of energy restriction on performance is not surprising.

We found trends that indicated the performance of CrS was improved relative to Pl after energy restriction. The mean performance of CrS in test four improved from test three in all measures, while that of Pl diminished. These changes were not great enough to be significant, but the pattern is striking. Several studies measuring cycling performance in subjects in energy balance supplementing with Cr have found increases in total work during repeated sprint cycling (1,3,6,28,30). Although the performance benefits seen in our study did not prove significant, trends toward performance enhancement were seen in performance measures that have repeatedly been shown to benefit from Cr supplementation in energy balance.

The trends for improved performance of CrS relative to Pl follows the muscle Cr and CrP store data, since elevations in muscle Cr and CrP can have a direct effect on energy metabolism and performance during high-intensity, intermittent exercise. The ergogenic effect of Cr supplementation has been related to increases in the resynthesis rate of CrP during recovery, as a result of increased availability of free Cr and a subsequent ability to maintain [free Cr] above the K_m for CPK (6,13,21). As a result, CrP is more readily available to fuel subsequent bouts of high- or maximal-intensity exercise. Greenhaff et al. (13) found that CrP resynthesis rates were only affected 60 to 120s into the rest period after intense electrically evoked isometric contraction, after which muscle CrP levels were decreased to <10 mmol $\cdot$ kg^{-1}. However, it was suggested that exercise bouts resulting in smaller reductions in muscle CrP may have a similar stimulatory effect on CrP resynthesis even if the rest periods were shorter than 60s.

In contrast to our results, Ööpik et al. (25) found that Cr supplementation during energy restriction resulted in maintenance of rather than improvements in peak torque and work at peak torque, while consumption of a placebo resulted in performance decrements during maximal knee extensor exercise of 4.71 rad $\cdot$ s^{-1}. However, peak torque and work

at peak torque were not affected by energy restriction or supplementation at angular velocities of 1.57 or 3.14 rad · s^{-1}, and work during 45s of submaximal isokinetic knee extensions was significantly reduced after Cr supplementation. These results are difficult to compare with ours because of the differences between performance tests. However, the lack of significant performance benefit observed in the present study and by Ööpik et al. (25) suggest that the ergogenic effect of Cr supplementation may have been blunted due to energy restriction. The blunted effect of supplementation on performance may be related to the impairment of Cr uptake at the muscle. Both studies show that anaerobic performance may be enhanced during short-term energy restriction in athletes that supplement with Cr relative to athletes that do not.

Body Weight and Body Composition. Energy restriction caused a significant reduction in body weight with no effect of supplement (Fig. 1). Ööpik et al. (25) found a mean loss in body weight of 2.2 kg over 5 d of energy restriction with Cr supplementation, which was significantly less than the 3.3 kg lost in the same subjects supplementing with glucose. It is possible that the difference in the duration of energy restriction accounted for the lack of a significant supplementation group by time interaction in our study.

The motivation for athletes to reduce body weight is generally to reduce body fat, with maintenance of FFM (36). Studies using various exercise and dietary strategies have shown that maintenance of FFM during energy restriction is difficult. Heyward et al. (29) reported that substantial resistance training for 6 to 17 weeks did not prevent lean tissue loss during energy restriction in bodybuilders preparing for competition. Walberg et al. (36) found that bodybuilders consuming moderate protein and high carbohydrate (MP/HC) during 7 days of energy restriction were in negative nitrogen balance, while those consuming high protein and moderate carbohydrate (HP/MC) were in positive nitrogen balance. In our study, body weight, FFM, and %BF were significantly reduced in all subjects undergoing energy restriction. The percentage of FFM lost was greater in Pl than CrS, while the reductions in body weight and %BF were not influenced by supplementation.

In short-term, rapid weight loss, many or all FFM and body weight changes are the result of dehydration (9). Similarly, FFM and body weight changes seen with short-term

Cr supplementation during energy balance are largely the result of fluid retention, while %BF is not affected (12,21,22,34). In our study, the combined effects of rapid weight loss due to energy restriction and Cr supplementation resulted in a difference in the change in FFM between groups. This difference between groups was most likely due to decreased loss of fluid in CrS as a result of supplementation. It is surprising that a similar effect of supplementation on body weight was not seen. It is notable that the p-value for % change in body weight was 0.13. A longer period of energy restriction or a greater number of subjects may have caused body weight differences to differ between groups. Nonetheless, mean body weight reductions were greater in Pl, and mean %BF reductions were greater in CrS, but the changes were not significant.

Summary. This study has shown that energy restriction can result in significant losses in muscle Cr storage, and that Cr supplementation during this period can negate this effect and result in significant increases in muscle Cr and CrP storage. Although performance was not significantly altered, there were trends toward increased total work and work capacity in subjects supplementing with Cr relative to those supplementing with sucrose. Body weight and body fat were significantly reduced in all subjects undergoing energy restriction, but this reduction was not influenced by supplementation. The percent change in FFM was significantly less in subjects who supplemented with Cr than in those who supplemented with sucrose. Athletes that utilize energy restriction as a training strategy may benefit from Cr supplementation for the maintenance and enhancement of performance and the minimization of losses in FFM.

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Table 1. Subject Characteristics

Group	CrS	Pl	C
n	8	8	8
Age (yrs)	20.5±0.5	21.6±0.8	22.9±0.6
Weight (kg)	83.0±6.4	81.7±4.5	80.6±2.9
Height (cm)	178.8±2.2	176.4±1.9	178.1±1.3
Years Lifting	5.4±0.4	4.6±0.9	5.1±1.1

All values are mean ± SEM.

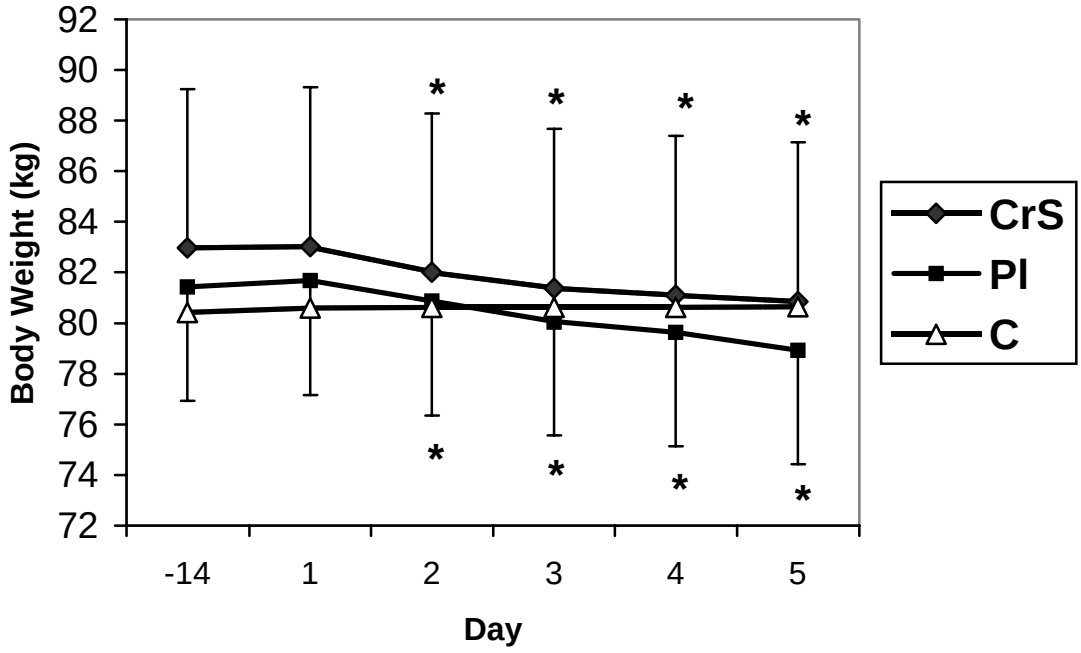

Figure 1. Mean body weight during the week.

*****Indicates significantly different from day 1 - CrS and Pl only ($p<0.05$).

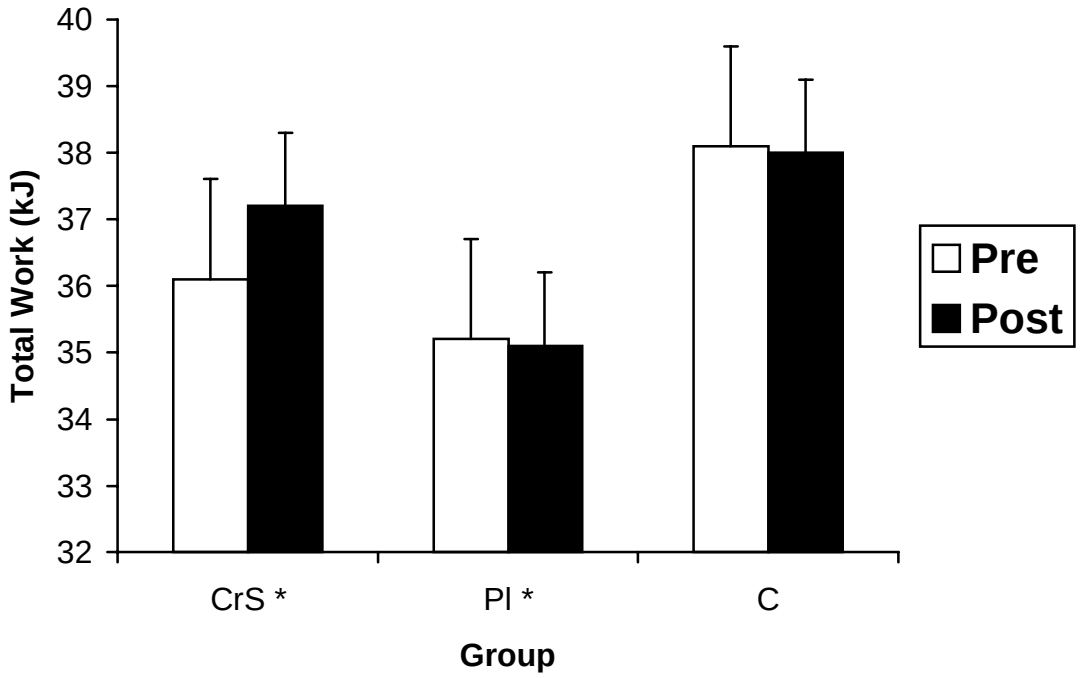

Figure 2. Total Work performed over all 10 sprints before and after experimental period.

* Indicates trend for group by time interaction (p=0.078)

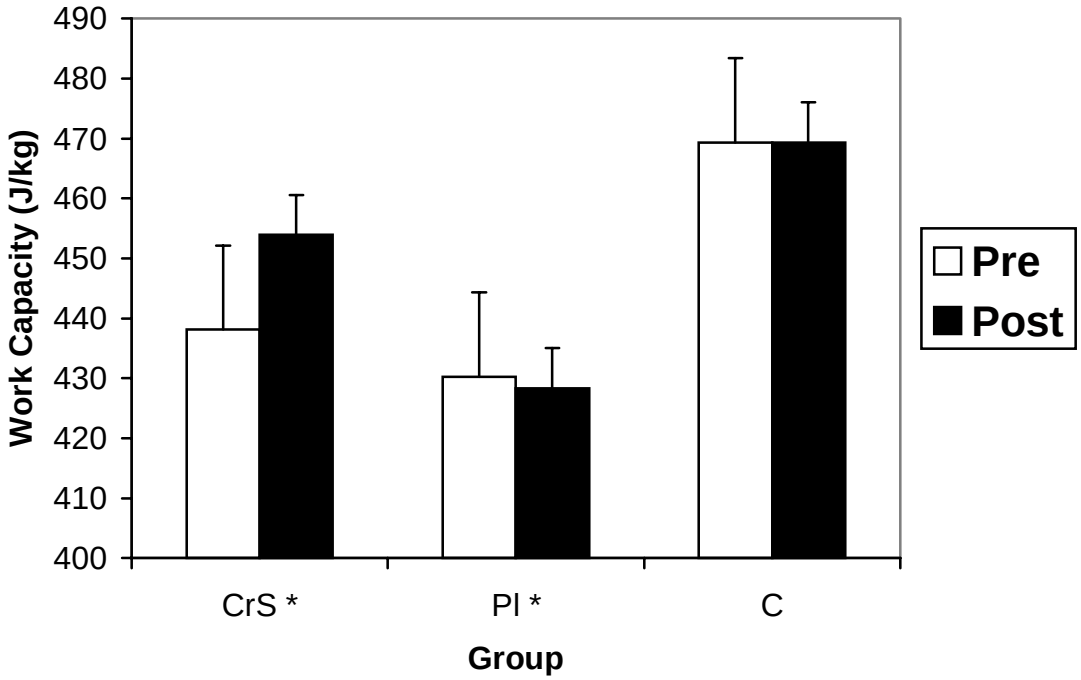

Figure 3. Work capacity before and after experimental period.

* Indicates trend for group by time interaction between CrS and Pl (p=0.058)

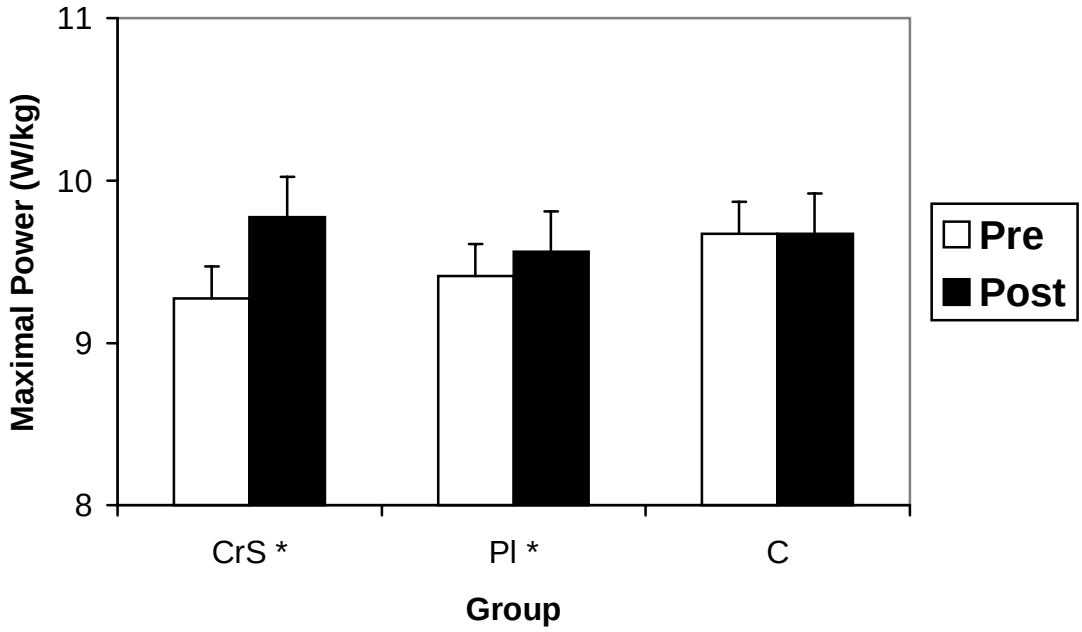

Figure 4. Maximal Power before and after experimental period

***** Indicates significant time effect for CrS and Pl combined ($p<0.05$)

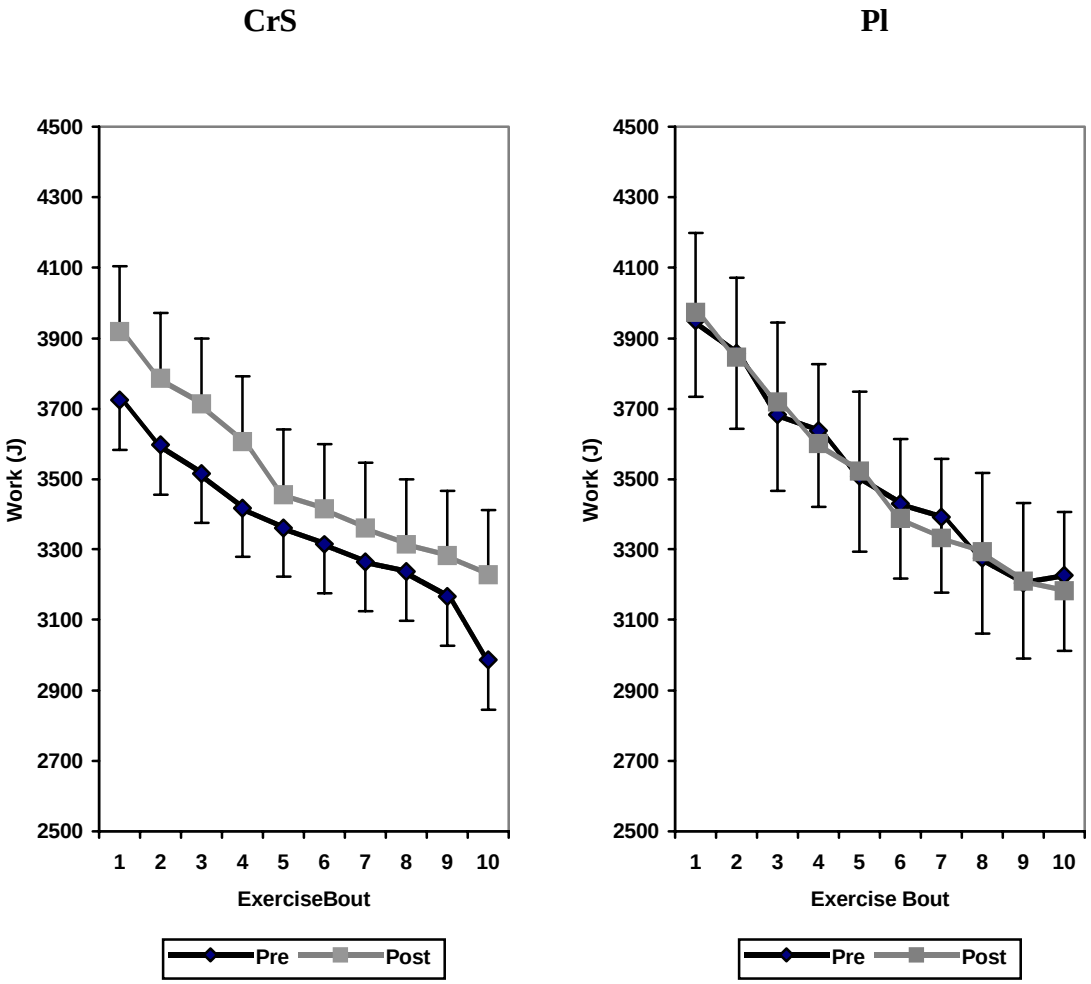

Figure 5. Total work performed during individual bouts of the 10 x 6s cycle sprints

Group x time interaction for exercise bout 4 ($p<0.05$)

Trends for group x time interaction for exercise bouts 2 ($p=0.086$),7 ($p=0.098$), and 10 ($p=0.091$)

Trends for time effect in CrS only in exercise bouts 3 ($p=0.067$) and 4 ($p=0.086$)

Table 2. Muscle Fuel Measures

	CrS Pre	CrS Post	Pl Pre	Pl Post
n	6	6	6	6
Free Cr †	45.8±1.6	53.4±2.2 *	53.5±1.6	49.0±1.8 *
CrP ‡	68.9±0.7	80.0±4.6 *	75.1±2.0	70.4±2.2
Total Cr †	114.6±1.4	133.4±4.8 **	128.6±1.4	119.4±2.2 **
ATP	19.9±0.9	20.1±1.4	21.4±1.0	20.7±0.6

All values are in mmol/kg dm. All values are mean ± SEM.

***** Indicates significant difference between pre and post value ($p<0.05$), ** ($p<0.01$)

‡ Indicates significant group by time interaction ($P<0.05$), † ($p<0.01$)

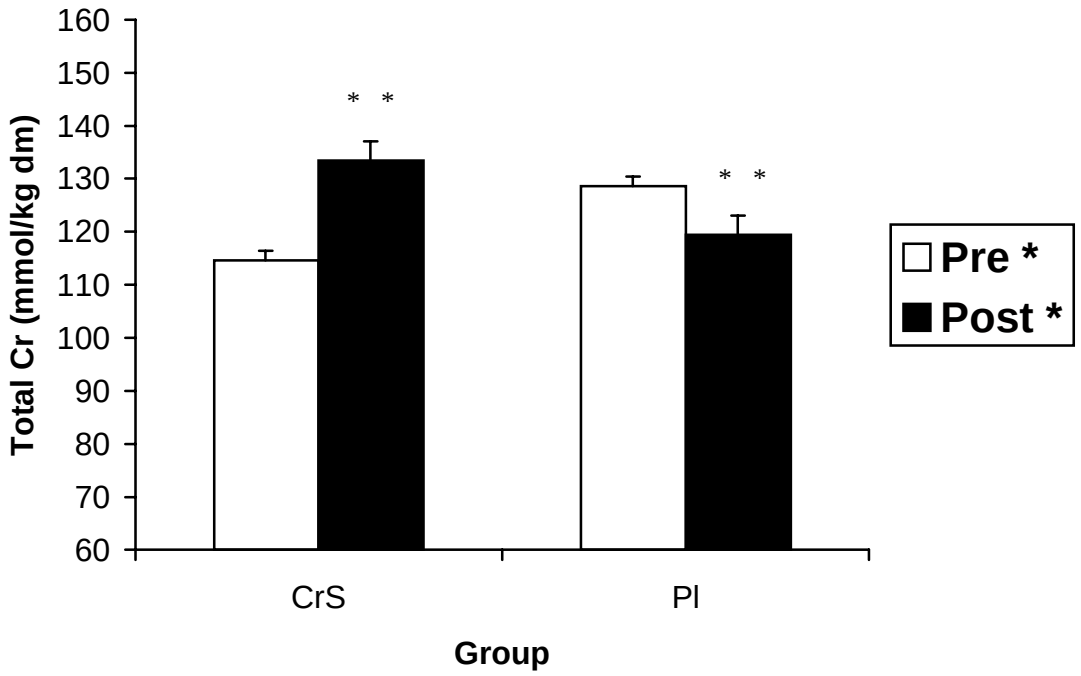

Figure 5. Total Cr

Significant group by time interaction ($p<0.01$)

* Indicates significant difference between groups ($p<0.01$)

* * Indicates significant change within group ($p<0.01$)

Table. 3 Body Composition Measures

	CrS Pre	CrS Post	Pl Pre	Pl Post	C Pre	C Post
n	8	8	8	8	8	8
% Body Fat	14.4±2.0	13.4±2.0 *	17.4±2.1	16.5±2.2 *	15.6±1.0	15.8±1.0
FFM (kg)	70.3±4.0	69.3±3.7 *	67.2±3.4	65.6±3.4 *	68.0±2.4	67.9±2.4

All values are mean ± SEM.

* Indicates significant difference between pre and post value ($p<0.01$)

CHAPTER IV SUMMARY AND RECOMMENDATIONS

Creatine supplementation is a widespread practice among athletes hoping to improve performance during intermittent, high-intensity exercise. Since athletes in many sports, including endurance based sports, engage in weight training to increase strength, Cr supplementation is popular with a wide range of athletes. It has been the subject of much debate in the scientific community and in the press. The revelation that such high-profile athletes as Mark McGwire, Brady Anderson, Mike Piazza and Shannon Sharpe use Cr has only increased its popularity.

One drawback to ingesting high levels of concentrated Cr is the lack of research regarding the long-term safety of the practice. Anecdotal claims in the media that Cr supplementation may be linked to seizures in 2 people, to the deaths of 3 collegiate wrestlers trying to make weight, to muscle cramping and tears, and to gastrointestinal distress have not been substantiated. Studies investigating the effects of supplementation periods longer than six months on liver and kidney function have not found any effects different than those associated with exercise training (34). Many researchers argue that since the body can synthesize Cr in the liver and kidneys, and there is a natural process for its breakdown and excretion either in the form of creatine or creatinine, the practice is safe. Also, the recommended supplementation protocol was designed to result in the rapid maximization of Cr stores followed by the maintenance of those stores. If the athlete follows this protocol, Cr intake above normal levels lasts only a few days (25,29). Nonetheless, more research is needed to show that supplementation and maintenance of Cr stores above normal levels will not have any detrimental long-term effect (34). As a result, it is recommended that anyone consuming Cr supplements do so only in the manner that has been used successfully in research, and that supplementation be stopped for at least 1 week every 2 to 3 months, to allow the body an adjustment period (56).

The initial 4 or 5 days of supplementation are referred to as the loading phase, because it is designed to load the muscles with the maximal amount of Cr possible. During the loading phase, 20 g/day Cr is consumed, a much higher level than is found in the average diet (18). Creatine consumption is spread over the course of each day and

should occur during a meal to promote maximal absorption at the gut. Once the muscle is Cr loaded, a maintenance dose is taken each day for the following month or so during the maintenance phase. During the maintenance phase, 2 to 5 g of Cr is consumed per day. This dose is slightly higher than the amount of Cr utilized each day in humans, roughly 2 g, coming from both endogenous and dietary sources.

Creatine supplementation has been shown to increase work production, strength, peak power output, and lifting volume during intermittent, high-intensity exercise as a result of increases in the muscular storage of Cr and CrP (2,6,10,22,33,34,38,43,47,54, 56). It also has been shown to result in increases in body mass and FFM. However, not all studies have resulted in performance enhancement. In general, studies where measurements involved single-effort exercise bouts or endurance exercise have not demonstrated performance enhancement (3,7,8,13,36,46,49,50,51).

Energy restriction is a strategy used by many athletes striving for improved performance through strength advantage in a weight class, reduced energy cost, or aesthetics. The wrestlers mentioned above all died while attempting to lose body weight to qualify for a low weight class by exercising under extreme conditions (two were wearing rubber suits), and by engaging in extreme energy deprivation. Energy restriction generally results in decreases in body weight, percent body fat, and FFM (27,35,57). It has also been shown to result in decreases in strength, endurance, peak torque, and work production (28,32,57,58,59). These changes in muscle function are likely to result in diminished performance in the athletic arena. Clearly, athletes considering energy restriction as a training strategy would seek to obtain the advantages of energy restriction without incurring impairments in muscle function. Also, it is generally desirable that losses in body mass be comprised of losses in body fat rather than FFM. Altering the diet is one strategy available to athletes in this situation (57).

Our study showed that muscle free and total Cr levels are significantly decreased after energy restriction for 4 d at 18 kcal/kg/day. There was no effect of the diet on CrP or ATP. It is difficult to determine if this change reflects the body's response to a Cr-free diet or to the energy restriction protocol or both. Harris et al. (25) reported that endogenous synthesis of Cr was sufficient to maintain muscle Cr levels, since two subjects

in the study were vegetarians with muscle Cr levels within the normal range. However, our study measured changes that occurred in the short-term as a result of a sudden change in kcal content of the diet. Greenhaff (18) reported that endogenous synthesis of Cr is depressed when Cr intake is high, but returns when Cr intake is low. The depressions in muscle Cr seen in Pl may reflect short-term responses that would eventually be reversed as a result of the body's adaptation to the diet by increasing endogenous synthesis of Cr.

Jeejeebhoy (32) did not measure muscle free Cr, but found a significant decrease in CrP as a result of severe energy restriction in rats and obese humans. It appears that the lack of effect of energy restriction on muscle CrP found in our study and that of Houston et al. (28) was due to the relative inseverity of the energy restriction protocols, which was markedly less drastic than that of Jeejeebhoy.

Supplementation with Cr during energy restriction negated the losses in muscle Cr seen in Pl, and resulted in significant increases in muscle Cr and CrP storage in CrS. This observation has not been previously reported. However, in energy balance, Cr and CrP have been shown increases as a result of a Cr supplementation protocol similar to that used in our study (19,25,29). The results of our study show that a similar effect occurs in subjects undergoing energy restriction.

Greenhaff et al. (19) and Harris et al. (25) noted that the increase in muscle Cr and CrP storage as a result of supplementation was more dramatic in subjects with low initial muscle Cr stores. Some subjects with high initial levels did not exhibit increases in muscle Cr as a result of supplementation, and were labeled non-responders (19). The energy restriction and formula diet may have resulted in a more dramatic elevation in muscle Cr and CrP in CrS since Pl demonstrated significant decreases in muscle Cr.

Performance in an intermittent, high-intensity cycling test was not significantly altered after energy restriction, although there were trends toward increased total work and work capacity in subjects supplementing with Cr relative to those supplementing with sucrose. Therefore, our results are in agreement with studies that have found increases in total work during repeated sprint cycling in subjects in energy balance supplementing with Cr (2,6,10,43,47). Our study found no effect of supplementation during energy restriction on peak power or maximal power, while Birch et al. (6) found increases in peak power in

Cr supplemented subjects in energy balance during cycling bout 1 of 3 repeated 30 s sprints. Ööpik et al. (39) found Cr supplementing subjects maintained peak torque and work at peak torque during energy restriction while subjects consuming a placebo did not.

The motivation for athletes to reduce body weight is generally to reduce body fat, with the maintenance of FFM (57). Body weight, body fat, and FFM have all been reported to be reduced during energy restriction (27,35,57). Therefore, dietary and exercise strategies have been developed to attempt to influence these changes so that FFM is maintained during weight loss. Heyward et al. (27) reported that substantial resistance training for 6 to 17 weeks did not prevent lean tissue loss during energy restriction in bodybuilders preparing for competition. Walberg et al. (57) found that bodybuilders consuming moderate protein and high carbohydrate (MP/HC) during 7 days of energy restriction were in negative nitrogen balance, while those consuming high protein and moderate carbohydrate (HP/MC) were in positive nitrogen balance. This effect of diet was not reflected in changes in body composition as measured by hydrostatic weighing, however, as the groups demonstrated similar decreases in % body fat and FFM.

Creatine supplementation has been associated with increases in body weight and FFM (2,10,19,33,34,47,53,55). Kelly et al. (33), and Kreider et al. (34) reported significant increases in body weight and FFM as a result of Cr supplementation, with no effects of supplementation on % body fat. Ööpik et al. (39) did not measure body composition, but found that subjects demonstrated greater losses in body weight when consuming a placebo than when supplementing with Cr during energy restriction. In our study, body weight and body fat were significantly reduced in all subjects undergoing energy restriction, but surprisingly, this reduction was not influenced by supplementation. However, the percentage of FFM lost by Pl was significantly greater than that lost by CrS. Although mean body weight reductions were greater in Pl, and mean body fat reductions were greater in CrS, there were no significant differences between groups. Therefore, our study is in agreement with Grindstaff et al. (22), who found significant improvements in swim times and arm ergometry work and power, even though body weight was not affected. Other studies that did not find changes in body mass as a result of Cr supplementation did not find performance benefits (41,50,51).

Creatine supplementation can result in increases in strength, work production, peak torque, and FFM, while energy restriction can result in decreases in these measures. The detrimental effects of energy restriction may be partially related to losses in the muscular storage of muscle Cr, and we showed that supplementation with Cr at this time can negate or even reverse this effect. We found that Cr supplementation caused a decreased loss in percentage of FFM in energy restricted athletes. However, Cr supplementation had no effect on changes in % BF or body weight during energy restriction. Therefore, we have shown that supplementing with Cr while simultaneously restricting kcal can result in a negation of some of the detrimental effects of energy restriction. Athletes using energy restriction as a training strategy for improving performance and maintaining FFM may benefit from including Cr supplementation in their dietary manipulations.

Recommendations for Future Research

Research in this area has been growing as a result of the huge popularity of Cr supplementation combined with the repeatability of studies showing performance enhancement. Still, questions exist as to the safety of ingesting Cr. Much remains to be discovered regarding this supplement.

1. We found that Cr supplementation during energy restriction resulted in significant increases in muscle Cr and CrP. We also found that energy restriction and consumption of a Cr-free diet resulted in significant decreases in muscle Cr, but not CrP. It would be beneficial to test if subjects undergoing energy restriction, but still consuming the dietary average of 2 $g \cdot d^{-1}$ demonstrate muscle Cr losses.
2. Our subjects were on a formula diet consisting of Ensure High Protein, Ross Laboratories, which is 54.7% carbohydrate, 21.3% protein, and 24.0% fat. Our laboratory has shown previously that macronutrient content of the diet during energy restriction can affect muscle function and nitrogen balance, but not changes in body composition (57). It is not known if the combined dietary manipulation of varying the macronutrient content of the diet and supplementing with Cr may have effects on performance or body composition.

3. It has been theorized that Cr supplementation may have a stimulatory effect on muscle protein synthesis, and long-term Cr supplementation has been shown to result in increases in FFM beyond those seen in the short-term. This effect is thought to be the result of increased intracellular water causing an increase in protein synthesis, increased muscle Cr levels causing an increase in protein synthesis, or increased muscle Cr levels allowing an increase in training volume. Future studies should investigate the effects of Cr supplementation alone and with training on protein synthesis.
4. Although Greenhaff et al. (19) determined that Cr supplementation increased the resynthesis rate of CrP during recovery, little is known about the cellular mechanism that causes a performance benefit. In vitro research into the effects of Cr supplementation on muscle function would give information about the mechanism that causes changes in performance.
5. It is thought that vegetarians may benefit from Cr supplementation more than carnivores, since they consume no dietary Cr. Harris et al. (25) reported that endogenous synthesis of Cr was sufficient to maintain muscle Cr levels, since two subjects in the study were vegetarians with muscle Cr levels within the normal range. However, Delanghe et al. (9) reported that vegetarians demonstrate lower levels of Cr in the blood and urine, but did not measure muscle Cr content. Therefore, it is still unknown whether or not consumption of a Cr-free diet alters muscle Cr storage.
6. The effect of dietary caffeine intake on performance during Cr supplementation is controversial. Vandenberghe et al. (53) reported that while increases in muscle CrP were similar among subjects that consumed caffeine and Cr simultaneously and those subjects supplementing only Cr, performance enhancement was only demonstrated by subjects consuming Cr alone. However, studies by Greenhaff et al. (19,20) and Birch et al. (6), found that Cr supplementation improved performance even though subjects ingested Cr after first mixing the supplement into coffee or tea. It would be beneficial to further investigate whether or not there is an interaction between dietary caffeine and Cr.

7. Currently, little is known regarding the effect of Cr supplementation on youth. There is some concern that young athletes who see professional athletes supplementing with Cr might take and abuse Cr and other supplements. It is not known if Cr supplementation would be beneficial to these young athletes, or have an adverse affect on long-term muscle growth.
8. Little research to date has investigated Cr supplementation and its potential therapeutic benefit. Gordon et al. (16) reported that chronic heart failure patients significantly improved leg strength after supplementing with Cr relative to patients that took a placebo, although there was no effect on ejection fraction. Sipilä et al. (44) found that low-dose Cr supplementation for 1 year resulted in an increase in the diameter of type II muscle fibers in the eyes of gyrate atrophy patients. Kreider et al. (34) reported that Cr supplementation for 4 weeks had a positive effect on the blood lipid profile of 25 collegiate football players. It is plausible that ingesting creatine may be beneficial to humans with muscle-wasting diseases, to elderly humans who have lost functionality due to loss of muscle tissue, or to active, hyperlipidemic individuals.
9. It is generally recommended that athletes supplementing with Cr consume 20 to 25 g $\cdot$ d^{-1} for 4 to 5 days to elevate muscle Cr. The same recommendation is made to athletes of all ages and sizes. Noonan et al. (38) found that athletes consuming 100 mg $\cdot$ kg^{-1} for 8 weeks improved performance in the 40 yard dash, while those consuming 300 mg $\cdot$ kg^{-1} did not. It is possible that athletes of different size and type may benefit from different supplementation protocols.
10. Many anecdotal reports have been made in the media that link Cr supplementation with muscle cramps, pulls, and tears. These generally come from athletic trainers and physicians that have noticed a dramatic increase in this type of injury as a result of the increased popularity of this supplement. Research into the connection between Cr supplementation and muscle pulls would help to determine strategies that could lead to the avoidance of these injuries.

APPENDIX A DETAILED DESCRIPTION OF TECHNICAL PROCEDURES AND RESEARCH METHODS

Subject Selection and Screening

Sixteen actively training male resistance trainers age 18 to 26 were selected. Subjects had trained for at least 2 years, 3 to 6 times per week, with no reported history of anabolic steroid use. Eight healthy control subjects of the same age were recruited separately. Subjects were screened for contraindications to weight loss and resistance exercise such as diabetes, heart conditions, orthopedic limitations and injuries, and major organ malfunctions. All subjects signed an informed consent form.

Preliminary Testing Procedures

Subjects who had been supplementing with Cr abstained for 30 days to allow muscle Cr levels to return to normal levels. Body weight maintenance prior to the start of the experimental period was determined by weighing subjects during the 2 weeks prior to the experimental period. On the preliminary test day, body weight was recorded again. Anyone with more than a 1.0 kg change over the 2 weeks was excluded. Body weight was assessed to the nearest 0.1 kg on a medical scale each day of the experimental period.

Subjects reported to the lab within 3 to 5 days of the experiment for 2 familiarization trials on the cycle ergometer. The familiarization tests were separated by at least 24 hours and were administered in the exact manner and under the same conditions as the experimental performance tests.

Subjects were randomly assigned to creatine supplement (Cr) and placebo (Pl) groups. Administration of Cr or Pl supplements was double-blind. Control subjects did not undergo energy restriction, supplementation, or muscle biopsy.

Experimental Conditions and Testing Protocol

All measurements were performed prior to and after a 4 d energy restriction period. The procedures for each measurement was identical for pre- and post-testing.

Subjects maintained their normal exercise routine of weightlifting 3 to 6 times per week throughout the experiment.

Performance tests were administered on a modified Monark cycle ergometer. Performance tests were conducted in the a.m., with subjects in the fasted condition. The cycle ergometer was integrated with a microcomputer for the purpose of determining power and work outputs, as described by Williams et al. (60). The ergometer was mounted on wooden boards and held down by 75 lb. weights for stability. The cycle was fitted with road-bike style handlebars and toe clips. A load cell attached to the friction belt of the ergometer flywheel allowed for the measurement of friction changes throughout each cycle sprint. Two magnets were attached to the cycle pedal crank 180° apart with epoxy. A magnetic switch was attached to the body of the ergometer so that one magnet passed close by the switch with each ½ pedal rotation. As a result of the placement of the magnets and the rotation of the pedal crank, 2 impulses were sent to the computer and recorded for each pedal rotation, so power output was computed for each ½ pedal revolution throughout each cycle sprint.

Body composition was measured via hydrostatic weighing, immediately after the performance test. Underwater weight was recorded after subjects submerged and exhaled fully. Residual volume was measured via the oxygen dilution technique (61). Underwater weight and residual volume were then used to calculate percent body fat for each subject.

Placebo and Cr subjects reported to the Va Tech athletic training room in the early afternoon of the preliminary testing day for muscle biopsies. A portion of the lateral portion of the vastus lateralis muscle was removed using the needle muscle biopsy technique with suction. A local anesthetic (lidocaine) was injected in several punctures following cleansing. After 4 to 5 minutes, an ~ ½ inch long and deep incision was made in the leg. The needle was inserted into the incision and a sample of muscle was removed. The sample was immediately frozen in liquid nitrogen and stored at -80° C until analysis for Cr, CrP, and ATP.

Energy restriction began with a 12 hour fast prior to the preliminary performance and body composition tests. For the next 4 d, Pl and Cr supplement groups consumed a formula hypoenergy diet of 18 kcal/kg/day. On the fifth day, subjects consumed ½ of their

daily ration of food after the performance and body composition tests and prior to the second muscle biopsy. The diet consisted entirely of Ensure, Ross Laboratories, which is 54.7% carbohydrate, 21.3% protein, and 24% fat. The subjects consumed no other Calories. Control subjects ate a normal unrestricted diet throughout the experiment.

Supplementation began after the preliminary muscle biopsy on day 1, and lasted until the final muscle biopsy on day 5. Therefore, the supplementation period was 4 days long, but included ½ of the first and last day. Creatine or Pl supplements were distributed to subjects in 4 packets to be taken at intervals throughout the day with food. Each Cr packet contained 5 g of Cr plus 1 g of sucrose, while each Pl packet contained 6 g of sucrose. The powder in each packet was mixed into 1 can of Ensure for consumption. On the first and last day, subjects received 2 supplement packets, after and prior to that day's muscle biopsy, respectively. On each of the intervening 3 days, subjects were given 4 supplement packets.

30 days prior to day 1 - End prior Cr supplementation
14 days prior to day 1 - Record subject weight
day 1 - Practice performance test
day 2 - off
day 3 - Repeat practice performance test
day 4 - off
day 5 - a.m. Record subject weight and compare to 2 weeks prior to assure weight maintenance (+/- 0.5 kg acceptable). Body composition test. Performance test 1. Begin energy restriction for P and Cr groups. Cr or Pl supplements 2 packets.
p.m. Needle muscle biopsy to assess muscle Cr and CrP levels
day 6 - Energy restriction. Cr or Pl supplements 4 packets
day 7 - Energy restriction. Cr or Pl supplements 4 packets
day 8 - Energy restriction. Cr or Pl supplements 4 packets
day 9 - a.m.: Body composition test. Performance test 2. Cr or Pl supplements 2 packets.
p.m. Needle muscle biopsy

Fig. 2 Schematic Illustration of Study Design

Measurement Procedures for the Repeated Sprint Cycling Performance Test

Subjects reported to the Muscular Function Laboratory in the a.m. in the fasted state. After body weight was recorded, a standard warm-up was performed. The warm-up consisted of 5 minutes of cycling at 0.5 kiloponds (kp) and 60 rpm on a standard Monark cycle ergometer. Two cycling periods of increased intensity followed the 5 minute cycling period. Both were 30 s in duration at a resistance of 1.5 kp: the first was at 85 rpm while the second was at 115 rpm. Following the warm-up, subjects had five minutes to stretch and prepare for the repeated sprint cycling test.

The performance test consisted of 10 maximal effort cycling sprints lasting 6 s each. Resistance during the maximal effort cycling bouts was 0.075 kg per kg body weight, hung from the friction belt around the flywheel. Subjects received verbal encouragement throughout each bout. Sprints separated by 30 s of passive recovery. Subjects remained seated throughout exercise and recovery. The feet were placed parallel to the ground for the start of each sprint, so that the first magnetic impulse was recorded uniformly for each sprint. Water was consumed ad-libitum during the passive recovery periods.

Measurement Procedures for Body Composition

Body composition was measured via the hydrostatic weighing method. Subjects exhaled fully and submerged themselves in a tank (Novel Products, Rockton, IL) while sitting on a chair attached to a load cell. The load cell was attached to a computer that calculated the subjects' underwater weight based on the load cell output. The 3 highest values obtained from 8 measurements were used.

Residual volume of the lungs was measured by the oxygen dilution technique (61). Subjects were fitted with a nosepiece and mouthpiece. A mouthpiece was attached to a rebreathing bag filled with 5 liters of oxygen. The mouthpiece was placed in the mouth of the subject and a clip was placed on the nose so that air could pass only through the mouth. Subjects breathed from the bag 5 times before the bag was sealed and analyzed for oxygen and carbon dioxide. The rebreathing test was repeated twice to ensure the repeatability of the lung volume measurement.

Measurement Procedures for Muscle Cr, CrP, and ATP Stores

Muscle samples taken before and after energy restriction and supplementation were measured for Cr, CrP, and ATP using enzymatic, spectrophotometric techniques. The enzymatic procedure was based on the equilibrium principle of enzyme reactions. By manipulating the substrates and cofactors of a reaction, it is possible to shift the equilibrium to a point where virtually all of the assayed substrate is consumed. The reaction of the assayed substrate is coupled with the reduction or oxidation of the pyridine nucleotides (NAD $\leftrightarrow$ NADH, NADP $\leftrightarrow$ NADPH). NADH and NADPH form peak absorbencies at wavelengths of 334 to 365 nm.

Upon removal from the leg, samples were frozen in liquid nitrogen while still in the biopsy needle. The muscle was removed by slightly warming the needle with a hand and a paper towel. The needle plunger and forceps were then used to place the sample in a cryo vial for freezing and storage at -80° C.

Freeze Drying

The samples were weighed while still frozen. This was considered the sample wet weight. Care was taken not to allow any thawing at any time during this procedure. After weighing the samples were placed in labeled 12 x 75 test tubes in a Styrofoam cup with liquid nitrogen in the bottom. The test tubes were covered with gauze held in place with a rubber band, and placed into the pre-cooled freeze dryer. A vacuum of 10-100 microns was applied, at a temperature of -30 to -40° C. Samples were left to freeze dry for at least 12 hours. Upon removal from the freeze dryer, samples were placed in a sealed desiccant container and left to come to room temperature. Once at room temperature, the sample dry weight was measured, and the wet to dry ration was calculated. A ratio of 4 to 4.3 is expected.

Powdering Tissue

The samples were dissected to remove fat, blood, and connective tissue, and powdered. A petri dish, two sets of forceps, and a scalpel blade were cleaned with ethanol

and a muscle sample was placed into the petri dish. The sample was ground to smaller particles with forceps and pieces of fat, blood, and connective tissue were picked out until only muscle remained. Connective tissue looks white and feathery, and is stickier and tougher than muscle. Blood is a darker red than muscle and very light and crumbly. Once the powdered sample was dissected, it was put into a pile, transferred to a tared test tube, and weighed. This was the powder weight, or extraction mass. If the sample was large, the powder was separated into aliquots of greater than 3 mg (prefer at least 3.5 mg). If the powdered samples were not going to be analyzed that day, they were put into a sealed desiccant container and frozen at -80° C. When the powdered samples were defrosted for extraction and analysis, they were brought to room temperature inside a sealed desiccant container.

Extraction

Prior to extraction, the centrifuge was precooled to +4° C, and the neutralizing capability of the stored potassium bicarbonate ($KHCO_3$) was tested. Four ml of Perchloric Acid (PCA) were added to 1 ml of $KHCO_3$. The pH of this solution was measured after all bubbling stopped. If the pH was not between 6.5 and 7, fresh $KHCO_3$ was prepared.

Ten or fewer freeze-dried and powdered samples were brought to room temperature inside a sealed desiccant container. The volume of PCA in microliters (uL) added to each sample was equal to the muscle powder weight multiplied by 40. PCA was quickly added to the samples, which were in test tubes on ice. For five minutes, the samples plus acid were gently mixed to ensure the PCA reached all muscle. Care was taken to minimize muscle adherence to the sides of the test tube. The samples were then centrifuged at 5,000 rpm for 5 min at +4° C. The supernatant, which was kept on ice, was removed using Pasteur pipettes, transferred to tared 12 x 75 test tubes, and weighed. The weight of the supernatant (extract mass) was divided by 1.025, to give extract volume in uL, which was divided by 4 to calculate the volume in uL of $KHCO_3$ required to neutralize the PCA. The $KHCO_3$ was added and vortexed several times until bubbling stopped. The mixture was centrifuged at 5,000 rpm for 15 min at +4° C. The supernatant was removed

with Pasteur pipettes and transferred to 12 x 75 test tubes. Analysis for ATP, Cr, and CrP was always performed on the same day as extraction.

Spectophotometric Analysis of ATP, Cr, and CrP

For the analysis of muscle ATP, free Cr, and CrP levels, two separate assays were performed. One assay measured the amount of free Cr in the extract, while the other measured the amount of both ATP and CrP. Many of the reagent solutions required for both assays were mixed and stored several weeks prior to analysis, while other reagent solutions had to be mixed the same day the assay was performed.

Creatine Phosphokinase (CPK) enzyme was used in both assays. A solution of 0.5% $NaHCO_3$ and 0.05% Bovine Serum Albumin (D5) was prepared and stored at +4° C for use in the CPK solution. Lyophilized CPK was mixed with D5 at a ratio of 17 mg per ml for use in the assays.

Reagent solutions that were mixed and stored for the ATP and CrP assay were 0.05 M DTT (F1, -20° C), 0.01 M ADP (F3, -20° C), 0.125 M glucose (F4, -20° C), and 1M Triethanolamine mixed with 0.1 M Magnesium Acetate and 0.01 M EDTA at a pH of 7.5-7.6 (D1, +4° C). Solutions of 0.025 M NADP (F2), and Hexokinase (HK) enzyme diluted in a 1:1 ratio with water were prepared on the day of analysis.

After extraction, the ATP and CrP assay reagent was mixed. Five Hundred microliters of D1, 100 uL of F1, 200 uL of F2, 20 uL of F3, 200 uL of F4, 3,980 uL of water, and 17 uL of Glucose-6-Phosphate Dehydrogenase were mixed in a test tube. The assay was run in 330 uL cuvettes that were placed in a spectrophotometer set at a wavelength of 340 nanometers (nm). Twenty-five uL of muscle extract was added to a cuvette, followed by 225 uL of ATP/CrP reagent. The solution was then mixed with a stirring rod, and the background absorbance was monitored for > 1 min. For the measurement of ATP, 3.6 uL of diluted HK were added to the cuvette and mixed. Changes in absorbance were monitored each minute for the next 5 to 6 minutes. When there was no longer any change in absorbance, 2.25 uL of the CPK/D5 solution was added to the cuvette and mixed. Changes in absorbance were monitored each minute for the

next 10 to 15 minutes. When there were no longer any changes in absorbance, the reaction was completed.

Reagent solutions that were mixed and stored for the free Cr assay were 2 Molar (M) KCl (labeled D3, stored at +4° C) and 0.025 M PEP-tri-cyclohexyl-ammonium salt (F6, -20° C). On the day of analysis and prior to the extraction, solutions of 0.025 M ATP (F5), 0.012 M NADH (F7), and 0.32 M Glycine mixed with 0.016 M Magnesium Acetate at a pH of 9.0-9.1 (D4) were prepared.

After extraction, the free Cr assay reagent was mixed. Two thousand microliters of D4, 100 uL of D3, 400 uL of F5, 300 uL of F6, 90 uL of F7, 3100 uL of water, 6 uL of Lactate Dehydrogenase enzyme, and 4.9 uL of Pyruvate Kinase enzyme were mixed in a test tube. The assay was run in 330 uL cuvettes that were placed in a spectrophotometer set at a wavelength of 340 nm. Twenty uL of muscle extract was added to a cuvette, followed by 300 uL of free Cr reagent. The solution was then mixed with a stirring rod, and the background absorbance was measured. The measurement of free Cr proceeded with the addition and mixture of 11.3 uL of the CPK - D5 solution. Changes in absorbance were monitored for the next 10 to 12 minutes. Since this reaction can drift after about 7 min, absorbance changes were compared each minute, and the point where the absorbance change minute became constant was considered the end of the reaction.

Calculation of Muscle ATP, Cr, and CrP in mmol/kg

Knowing the changes in absorbance resulting from changes in NADH or NADPH levels, it is possible to determine the metabolite concentration in the muscle sample through a calculation (Fig. 3). These absorbance changes are related to the amounts of ATP, Cr, or CrP that were found in the muscle sample. First, a dilution factor (DF) is determined, where DF= ((mg dry muscle + uL PCA added) / mg dry muscle) * 1.25, and 1.25 is a constant determined by (uL PCA + uL $KHCO_3$) / uL PCA. For the purposes of this study, DF was always 51.3, since the uL of PCA added was always 40 times the dry muscle weight in mg.

$$\text{mmol/kg dm} = (\Delta A * TV / E * SV) * DF$$

ΔA = Change in absorbance measured in the spectrophotometer

TV = Total volume in cuvette in uL

E = Extinction coefficient or molar absorption coefficient – always 6.22 cm^2/mmol at 340 nm

SV = Sample Volume

DF = Dilution factor, defined above – always 51.3 for the purposes of this study

Fig. 3 Calculation of Metabolite Concentrations

Data on Reliability and Sensitivity of Important Dependent Measures

The reliability of the performance test was assessed by correlating data from tests 2 and 3. The correlation for total work performed over all 10 sprints was r=0.89, and for peak power was r=0.86. After completion of test 3, C performed the fourth test 4 days later without undergoing energy restriction or supplementation of any kind. The correlation between tests 3 and 4 for C for total work, peak power, total work fatigue ratio, and peak power fatigue ratio was r=0.99, r=0.99, r=0.85, and r=0.90 respectively.

APPENDIX B RAW DATA TABLES

Table 1. Individual Subject Characteristics

Subject #	Group	Age (years)	Weight (kg)	Height (cm)	Yrs. Lifting
1	Cr	20	74	175	7
2	Cr	19	77	173	6
3	Cr	19	95	185	6
6	Cr	20	80	178	6
10	Cr	21	68	170	4
12	Cr	20	72	177	4
13	Cr	23	123	188	6
14	Cr	22	76	184	4
4	Pl	22	76	174	2
5	Pl	22	92	180	3
7	Pl	22	65	170	7
8	Pl	19	76	169	3
9	Pl	26	91	182	9
11	Pl	23	77	173	3
15	Pl	20	104	181	6
16	Pl	19	74	182	4
17	C	20	76	176	5
18	C	21	79	174	9
19	C	24	71	176	1
20	C	22	89	179	6
21	C	23	88	182	6
22	C	23	92	177	8
23	C	25	72	176	0
24	C	25	79	185	6

All values are mean ± SEM.

Table 2. Individual Subject Body Weight Data

Subj	Group	2wk	Mon	Tues	Wedn	Th	Fri	Change	%Chng
1	Cr	75	74.1	72.7	73.1	73.2	72.7	-1.4	1.88934
2	Cr	76	76.5	74.5	74.4	74.7	75.5	-1	1.30719
3	Cr	94.7	95	94.5	93.5	93.1	92.3	-2.7	2.84211
6	Cr	79.2	79.5	78.7	77.3	76.9	76.6	-2.9	3.6478
10	Cr	67.2	67.8	68	67.6	67.4	67	-0.8	1.17994
12	Cr	72.5	72	71.2	70.5	70.5	70.3	-1.7	2.36111
13	Cr	122.3	123.2	122.1	120.8	120	119.8	-3.4	2.75974
14	Cr	76.7	76	74.2	73.8	73	72.5	-3.5	4.60526
4	Pl	74.5	75.5	75.3	75	74.5	73.5	-2	2.64901
5	Pl	90.8	92	91.3	90.6	89.6	89.4	-2.6	2.82609
7	Pl	65.2	64.5	64.5	62.8	62.5	62	-2.5	3.87597
8	Pl	75.5	75.6	75.5	74.3	74	73	-2.6	3.43915
9	Pl	90.1	91	89.5	89	89	87.8	-3.2	3.51648
11	Pl	77	76.8	75.2	73.9	73.5	72.6	-4.2	5.46875
15	Pl	104.5	104	103.3	102.8	102.5	101.8	-2.2	2.11538
16	Pl	73.8	74	72.3	72.1	71.5	71.3	-2.7	3.64865
17	C	75.3	76				75.9	-0.1	0.13158
18	C	78.4	78.6				78.5	-0.1	0.12723
19	C	71	71				72	1	-1.4085
20	C	89.9	89.2				89.2	0	0
21	C	87.4	87.7				87.7	0	0
22	C	91	91.9				92.1	0.2	-0.2176
23	C	71.5	71.5				71	-0.5	0.6993
24	C	78.7	78.7				78.6	-0.1	0.12706

All values are mean kg ± SEM.

Table 3. Individual Subject Body Composition Data

Subj	Group	BCpre (%)	BCpst (%)	Change	FFMpre (kg)	FFMpst (kg)	Change	%Chng
1	Cr	16.043	16.0241	0.0188	61.7084	61.0505	0.6579	1.06623
2	Cr	13.3654	12.7125	0.6529	66.2754	65.9021	0.3733	0.56339
3	Cr	15.7815	15.2941	0.4873	80.0076	78.1836	1.8241	2.27986
6	Cr	16.0662	13.097	2.9692	66.7274	66.5677	0.1596	0.23926
10	Cr	6.5975	5.8261	0.7714	63.3269	63.0965	0.2303	0.36376
12	Cr	15.9253	14.8891	1.0361	60.5338	59.8329	0.7008	1.15783
13	Cr	24.0176	23.482	0.5356	93.6103	91.0564	2.5538	2.72817
14	Cr	7.3732	5.6719	1.7012	70.3964	68.3878	2.0085	2.85319
4	Pl	24.0596	23.6884	0.3711	57.335	56.089	1.246	2.17318
5	Pl	21.446	20.9638	0.4822	72.2697	70.6584	1.6113	2.22959
7	Pl	9.6725	8.1562	1.5163	58.2612	56.9432	1.3181	2.26234
8	Pl	22.4905	21.6889	0.8016	58.5972	57.1671	1.4301	2.44052
9	Pl	12.3092	11.5537	0.7555	79.7986	77.6559	2.1427	2.68519
11	Pl	17.1262	15.2107	1.9155	63.6471	61.557	2.09	3.28380
15	Pl	22.3947	21.5057	0.889	80.7095	79.9072	0.8023	0.99407
16	Pl	9.6137	9.1832	0.4304	66.8859	64.7523	2.1335	3.18979
17	C	16.9013	17.4312	-0.5298	63.155	62.6697	0.4852	0.76836
18	C	18.0179	18.1027	-0.0847	64.4379	64.2894	0.1485	0.23045
19	C	13.6834	14.232	-0.5486	61.2848	61.7529	-0.4681	-0.7639
20	C	16.8316	16.8922	-0.0606	74.1862	74.1322	0.054	0.07286
21	C	9.8356	10.2695	-0.4339	79.0742	78.6936	0.3805	0.48124
22	C	17.1192	17.664	-0.5448	76.1674	75.8314	0.336	0.44113
23	C	19.8443	19.7645	0.0797	57.3113	56.9672	0.3441	0.60049
24	C	12.739	12.3519	0.3871	68.6744	68.8914	-0.217	-0.316

All values are mean ± SEM.

Table 4. Individual Subject Performance Data - Work

Subj	Group	Twfpre (%)	Twfpost (%)	TWpre (kJ)	TWpost (kJ)	Work Cap pre (J/kg)	Work Cap post (J/kg)
1	Cr	9.04	8.75	35.41	35.5	477.9	479.1
2	Cr	15.59	19.92	32.48	33.25	424.6	434.6
3	Cr	22.26	21.35	37.21	43.58	391.7	458.7
6	Cr	22.56	22.42	34.41	34.86	432.8	438.5
10	Cr	16.28	21.34	29.63	29.7	437.0	438.1
12	Cr	19.68	16.32	29.1	29.41	404.2	408.5
13	Cr	21.77	18.1	52.69	51.4	427.7	417.2
14	Cr	6.6	12.89	37.89	39.51	498.6	519.9
4	Pl	15.63	13.57	31.26	31.75	414.0	420.5
5	Pl	22.88	15.57	41.04	40.64	446.1	441.7
7	Pl	12.12	22.43	26.99	27.21	418.4	421.9
8	Pl	31.52	27.64	31.23	30.72	413.1	406.3
9	Pl	12.42	10.54	39.17	38.53	430.4	423.4
11	Pl	12.12	19.38	31.67	31.3	412.4	407.6
15	Pl	13.87	14.38	46.16	46.39	443.8	446.1
16	Pl	23.98	29.09	34.33	33.96	463.9	458.9
17	C	13.14	16.26	28.27	29.92	372.0	393.7
18	C	13.98	15.25	34.01	34.11	432.7	434.0
19	C	15.69	22.52	37.37	36.31	526.3	511.4
20	C	15.48	14.7	49.44	48.69	554.3	545.9
21	C	16.39	15	44.62	44.85	508.8	511.4
22	C	15.51	17.52	44.94	43.7	489.0	475.5
23	C	22.7	19.34	28.47	28.34	398.2	396.4
24	C	3.16	2.37	37.28	38.28	473.7	486.4

All values are mean ± SEM.

Pre/Pst = Pre or Post energy restriction

PPf = Peak Power fatigue ratio

Twf = Total work fatigue ratio

PP = Peak Power

TW = Total Work

Table 5. Individual Subject Performance Data - Power

Subj	Group	PPfpre (%)	PPfpost (%)	PPpre (W)	PPpost (W)	MaxP pre (W/kg)	MaxP post (W/kg)
1	Cr	9.31	5.5	738.45	707.06	9.9	9.7
2	Cr	15.26	19.22	669.04	702.21	8.7	9.3
3	Cr	22.1	16.6	794.41	923.53	8.4	10.0
6	Cr	20.69	16.7	753.99	754.6	9.5	9.9
10	Cr	23.38	22.16	664.11	662.16	9.8	9.9
12	Cr	19.94	15.99	625.35	616.03	8.7	8.8
13	Cr	20.02	17.93	1,086	1,075.5	8.8	9.0
14	Cr	5.92	8.93	759.07	790.14	10.0	10.9
4	Pl	18.58	16.92	687.49	678.11	9.1	9.2
5	Pl	24.21	15.25	900.04	845.13	9.8	9.5
7	Pl	13.87	25.34	617.14	616.22	9.6	9.9
8	Pl	31.59	21.97	746.68	691.65	9.9	9.5
9	Pl	13.41	12.16	825.1	779.83	9.1	8.9
11	Pl	11.34	19.46	657.56	682.34	8.6	9.4
15	Pl	14.2	12.4	954.76	966.66	9.2	9.5
16	Pl	22.74	31.28	749.56	757.32	10.1	10.6
17	C	13.49	17.4	602.52	607.23	7.9	8.0
18	C	16.17	16.62	710.53	714.3	9.0	9.1
19	C	15.34	21.65	794.41	780.47	11.2	10.8
20	C	14.59	14.85	1,001.6	994.52	11.2	1101
21	C	12.59	14.54	883.1	909.59	10.1	10.4
22	C	12.88	13.02	915.59	913.05	10.1	9.9
23	C	18.39	20.26	614.99	620.43	8.6	8.7
24	C	6.15	5.35	735.48	728.54	9.3	9.3

Table 6. Individual Subject Muscle Fuel Store Data

Subj	Group	FreeCr Pre	FreeCr Post	CrP Pre	CrP Post	TotCr Pre	TotCr Post	ATP Pre	ATP Post
1	Cr	45.865	59.956	66.146	61.445	112.01	121.401	18.186	17.32
2	Cr	43.378	44.76	68.909	75.754	112.287	120.514	16.99	16.289
3	Cr	41.997	54.775	70.641	92.497	112.637	147.272	22.557	26.31
6	Cr	48.282	54.568	69.857	90.682	118.14	145.25	18.928	20
10	Cr								
12	Cr	43.033	49.388	69.898	78.187	112.931	127.575	21.444	20.537
13	Cr								
14	Cr	52.012	56.917	67.671	81.734	119.684	138.65	21.279	20.372
4	Pl								
5	Pl	52.238	53.825	81.984	64.805	134.221	118.63	22.288	19.363
7	Pl	51.272	43.336	78.764	79.053	130.036	122.389	25.196	22.763
8	Pl	58.022	53.325	70.971	74.311	128.992	127.635	21.774	22.681
9	Pl	57.758	51.824	69.871	68.965	127.63	120.789	19.569	19.404
11	Pl								
15	Pl	48.282	45.934	77.074	68.29	125.356	114.224	21.238	20.083
16	Pl	53.256	45.727	71.919	67.094	125.175	112.821	18.351	20.165

All values are mmol/kg dm. All values are mean ± SEM.

Empty lines represent subjects whose biopsies were unusable.

APPENDIX C STATISTICAL PROCEDURES AND ANOVA TABLES

The body weight, body composition, performance and muscle fuel store data were analyzed using a two-way analysis of variance (ANOVA) with repeated measures, with tests for simple effects. A tukey post-hoc procedure was used to establish simple interactions when main effects were observed. Statistical significance was set at alpha < 0.05.

Table 7. ANOVA table comparing pre-experimental subject characteristics

	DF	SS	MS	F	P
Age					
Between Groups	2	22.583	11.292	3.172	0.063
Within Groups	21	74.75	3.56		
Total	23	97.333			
Weight					
Between Groups	2	23.841	11.92	0.064	0.938
Within Groups	21	3,895.239	185.488		
Total	23	3,919.08			
Height					
Between Groups	2	24.25	12.125	0.436	0.652
Within Groups	21	584.25	27.821		
Total	23	608.5			
Years Lifting					
Between Groups	2	2.333	1.167	0.203	0.818
Within Groups	21	120.625	5.744		
Total	23	122.958			

Table 8. Two-way repeated measures ANOVA table comparing body weight at two weeks prior and day 1 – all groups

Source of Variation	DF	SS	MS	F	P
Group	2	50.113	25.056	0.069	0.934
Error	21	7,671.147	365.2943		
Time	1	0.317	0.317	1.589	0.221
Group*Time	2	0.072	0.036	0.179	0.838
Error	21	4.187	0.199		

Table 9. Two-way repeated measures ANOVA table for body weight each day of the week

	DF	SS	MS	F	P
Source of Variation	1	50.17	50.17	0.035	0.854
Group	14	20,125	1,437.557		
Time	5	79.728	15.946	47.081	<0.001
Group*Time	5	1.427	0.285	0.843	0.524
Error	70	0.339			
Time @ CrS	5	35.114	7.023	17.283	<0.001
Error	35	14.221	0.406		
Time @ Pl	5	46.042	9.208	33.974	<0.001

Table 10. Two-way repeated measures ANOVA table for body weight days 1 and 5 – all groups

Source of Variation	DF	SS	MS	F	P
Group	2	23.927	11.963	0.033	0.968
Error	21	7,637.16	363.674		
Time	1	31.687	31.687	104.505	<0.001
Group*Time	2	17.495	8.747	28.849	<0.001
Error	21	6.367	0.303		
Time @ CrS	1	18.923	18.923	32.485	0.001
Error	7	4.078	0.583		
Time @ Pl	1	30.25	30.25	129.116	<0.001
Error	7	1.64	0.234		
Time @ C	1	0.001	0.001	0.108	0.752
Error	7	0.65	0.093		

Table 11. One-way ANOVA table for percent change in body weight days 1 and 5

	DF	SS	MS	F	P
Between Groups	1	3.016	3.016	2.55	0.133
Within Groups	14	16.56	1.183		
Total	15	19.576			

Table 12. Two-way repeated measures ANOVA table for **%** body fat – all groups

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	75.784	37.892	0.711	0.503
Error	21	1,119.547	53.312		
Time	1	3.853	3.853	18.071	<0.001
Group*Time	2	3.716	1.858	8.714	0.002
Error	21	4.477	0.213		
Time @ CrS	1	4.175	4.175	9.772	0.017
Error	7	2.99	0.427		
Time @ Pl	1	3.206	3.206	21.207	0.002
Error	7	1.058	0.151		
Time @ C	1	0.188	3.074	3.074	0.123
Error	7	0.429	0.061		

Table 13. One-way ANOVA table for percent change in % body fat

	DF	SS	MS	F	P
Between Groups	1	28.283	28.283	0.615	0.446
Within Groups	14	644.341	46.024		
Total	15	672.624			

Table 14. Two-way repeated measures ANOVA table for fat-free mass – all groups

Source of Variation	DF	SS	MS	F	P
Group	2	92.735	46.368	0.26	0.774
Error	21	3,749.016	178.525		
Time	1	10.403	10.403	51.849	<0.001
Group*Time	2	4.391	2.196	10.943	0.001
Error	21	4.213	0.201		
Time @ CrS	1	4.525	4.525	10.6	0.014
Error	7	2.988	0.427		
Time @ Pl	1	10.199	10.199	84.515	<0.001
Error	7	0.845	0.121		
Time @ C	1	0.071	0.071	1.299	0.299
Error	7	0.381	0.054		

Table 15. One-way ANOVA table for percent change in fat-free mass

	DF	SS	MS	F	P
Between Groups	1	4.007	4.007	4.876	0.044
Within Groups	14	11.504	0.822		
Total	15	15.511			

Table 16. Two-way repeated measures ANOVA table for peak power

Source of Variation	DF	SS	MS	F	P
Group	1	8,631.149	8,631.149	0.436	0.52
Error	13	257,218	19,786		
Time	1	78.43	78.43	0.086	0.774
Group*Time	1	2,520.977	2,520.977	2.765	0.12
Error	13	11,854.3	911.866		

Table 17. One-way ANOVA table for percent change in peak power

	DF	SS	MS	F	P
Between Groups	1	77.867	77.867	2.617	0.13
Within Groups	13	386.758	29.751		
Total	14	464.626			

Table 18. Two-way repeated measures ANOVA for maximal power

Source of Variation	DF	SS	MS	F	P
Group	1	0.011	0.011	0.019	0.891
Error	13	7.183	0.553		
Time	1	0.798	0.798	5.884	0.031
Group*Time	1	0.231	0.231	1.706	0.214
Error	13	1.764	0.136		

Table 19. One-way ANOVA table for percent change in maximal power

	DF	SS	MS	F	P
Between Groups	1	58.14	58.14	1.65	0.221
Within Groups	13	458.016	35.232		
Total	14	516.156			

Table 20. Two-way repeated measures ANOVA table for total work

Source of Variation	DF	SS	MS	F	P
Group	1	3.899	3.899	0.066	0.802
Error	13	773.269	59.482		
Time	1	2.752	2.752	2.24	0.158
Group*Time	1	4.494	4.494	3.659	0.078
Error	13	15.968	1.228		

Table 21. One-way ANOVA table for percent change in total work

	DF	SS	MS	F	P
Between Groups	1	67.591	67.591	3.834	0.072
Within Groups	13	229.186	17.63		
Total	14	296.777			

Table 22. Two-way repeated measures ANOVA table for work capacity

Source of Variation	DF	SS	MS	F	P
Group	1	2085.299	2085.299	1.357	0.265
Error	13	19,970.5	1536.195		
Time	1	356.915	356.915	2.617	0.13
Group*Time	1	590.484	590.484	4.330	0.058
Error	13	1772.645	136.357		

Table 23. One-way ANOVA table for percent change in work capacity

	DF	SS	MS	F	P
Between Groups	1	67.591	67.591	3.834	0.072
Within Groups	13	229.186	17.63		
Total	14	296.777			

Table 24. Two-way repeated measures ANOVA table for peak power fatigue index

Source of Variation	DF	SS	MS	F	P
Group	1	76.903	76.903	1.168	0.3
Error	13	856.286	856.286		
Time	1	2.011	2.011	0.098	0.759
Group*Time	1	9.432	9.432	0.461	0.509
Error	13	265.754	20.443		

Table 25. One-way ANOVA table for percent change in peak power fatigue index

	DF	SS	MS	F	P
Between Groups	1	1,009.507	1,009.507	0.622	0.444
Within Groups	13	21,094.5	1,622.651		
Total	14	22,104			

Table 26. Two-way repeated measures ANOVA table for total work fatigue index

Source of Variation	DF	SS	MS	F	P
Group	1	23.805	23.805	0.339	0.570
Error	13	913.171	70.244		
Time	1	12.387	12.387	0.969	0.343
Group*Time	1	0.588	0.588	0.046	0.834
Error	13	166.22	12.786		

Table 27. One-way ANOVA table for percent change in total work fatigue index

	DF	SS	MS	F	P
Between Groups	1	147.257	147.257	0.094	0.764
Within Groups	13	20,437.3	1572.097		
Total	14	20,584.5			

Table 28. Two-way repeated measures ANOVA table for free Cr

Source of Variation	DF	SS	MS	F	F-av	P
Group	1	16.445	16.445	0.539		0.48
Error	10	305.207	30.521			
Group @ pre	1	177.8	177.8	8.96	4.49	
Group @ post	1	58.1	58.1	2.93	4.49	
Error	16		19.84			
Time	1	14.944	14.944	1.634		0.23
Group*Time	1	219.938	219.938	24.048		0.001
Error	10	91.457	9.146			
Time @ CrS	1	174.8	174.8	14.8		0.012
Error	5	59.08	11.8			
Time @ Pl	1	60.111	60.111	9.2		0.029
Error	5	32.38	6.5			

Table 29. Two-way repeated measures ANOVA table for CrP

Source of Variation	DF	SS	MS	F	F-av	P
Group	1	17.205	17.205	0.327		0.58
Error	10	525.527	52.553			
Group @ pre	1	117.2	117.2	2.57	4.35	
Group @ post	1	278.3	278.3	6.1	4.35	
Error	20		45.6			
Time	1	63.7	63.7	1.654		0.227
Group*Time	1	378	378	9.81		0.011
Error	10	385.5	38.55			
Time @ CrS	1	376.067	376.067	7.603		0.04
Error	5	247.323	49.465			
Time @ Pl	1	65.635	65.635	2.375		0.184
Error	5	138.165	27.633			

Table 30. Two-way repeated measures ANOVA table for total Cr

Source of Variation	DF	SS	MS	F	F-av	P
Group	1	0.0086	0.0086	0		0.991
Error	10	637.084	63.708			
Group @ pre	1	583.8	583.8	32.8	4.41	
Group @ post	1	590.3	590.3	32.8	4.41	
Error	18		48.4			
Time	1	140.4	140.4	4.246		0.066
Group*Time	1	1,174.5	1,174.5	35.5		<0.001
Error	10	330.8	33.08			
Time @ CrS	1	1,063.58	1,063.58	19.79		0.007
Error	5	268.716	53.748			
Time @ Pl	1	251.371	251.371	20.27		0.006
Error	5	62.006	12.401			

Table 31. Two-way repeated measures ANOVA table for ATP

Source of Variation	DF	SS	MS	F	P
Group	1	6.682	6.682	0.621	0.449
Error	10	107.537	10.754		
Time	1	0.263	0.263	0.15	0.707
Group*Time	1	1.215	1.215	0.692	0.425
Error	10	17.558	1.756		

Table 32. Two-way repeated measures ANOVA for Work within each 6s exercise bout

Source of Variation		DF	SS	MS	F	P
Group	Sprint 1	1	0.147	0.147	0.202	0.661
	2	1	0.192	0.192	0.284	0.603
	3	1	0.055	0.055	0.107	0.749
	4	1	0.086	0.086	0.135	0.719
	5	1	0.085	0.085	0.141	0.713
	6	1	0.015	0.015	0.026	0.874
	7	1	0.019	0.019	0.029	0.868
	8	1	0.048	0.048	0.001	0.978
	9	1	0.023	0.023	0.004	0.953
	10	1	0.072	0.072	0.116	0.739
Error	Sprint 1	13	9.49	0.73		
	2	13	8.775	0.675		
	3	13	6.642	0.511		
	4	13	8.24	0.634		
	5	13	7.857	0.604		
	6	13	7.455	0.573		
	7	13	8.593	0.661		
	8	13	7.945	0.611		
	9	13	8.261	0.635		
	10	13	8.049	0.619		
Time	Sprint 1	1	0.089	0.089	2.619	0.13
	2	1	0.06	0.06	2.789	0.119
	3	1	0.103	0.103	4.723	0.049
	4	1	0.044	0.044	2.665	0.127
	5	1	0.023	0.023	2.076	0.173
	6	1	0.065	0.065	0.508	0.489
	7	1	0.028	0.028	0.192	0.668
	8	1	0.017	0.017	0.965	0.344
	9	1	0.027	0.027	1.55	0.235
	10	1	0.073	0.073	1.604	0.228
Group*Time	Sprint 1	1	0.054	1.602	1.602	0.228
	2	1	0.075	0.075	3.443	0.086
	3	1	0.049	0.049	2.257	0.157
	4	1	0.093	0.093	5.648	0.034
	5	1	0.012	0.012	1.103	0.313
	6	1	0.039	0.039	3.032	0.105
	7	1	0.045	0.045	3.17	0.098
	8	1	0.066	0.066	0.383	0.547
	9	1	0.024	0.024	1.364	0.264
	10	1	0.152	0.152	3.339	0.091

Source of Variation		DF	SS	MS	F	P
Error	Sprint 1	13	0.44	0.034		
	2	13	0.282	0.022		
	3	13	0.283	0.022		
	4	13	0.215	0.017		
	5	13	0.143	0.011		
	6	13	0.166	0.013		
	7	13	0.186	0.014		
	8	13	0.225	0.017		
	9	13	0.229	0.018		
	10	13	0.591	0.045		
Time @ CrS	Sprint 1	1	0.132	0.132	2.727	0.15
	2	1	0.126	0.126	3.589	0.107
	3	1	0.138	0.138	4.987	0.067
	4	1	0.124	0.124	4.206	0.086
	5	1	0.032	0.032	1.509	0.265
	6	1	0.036	0.036	1.572	0.257
	7	1	0.033	0.033	1.35	0.289
	8	1	0.021	0.021	0.761	0.416
	9	1	0.048	0.048	2.01	0.206
	10	1	0.204	0.204	2.236	0.185
Error	Sprint 1	6	0.291	0.048		
	2	6	0.211	0.035		
	3	6	0.166	0.028		
	4	6	0.178	0.03		
	5	6	0.127	0.021		
	6	6	0.137	0.023		
	7	6	0.147	0.024		
	8	6	0.164	0.027		
	9	6	0.143	0.024		
	10	6	0.548	0.091		
Time @ Pl	Sprint 1	1	0.023	0.023	0.105	0.755
	2	1	0.04	0.04	0.04	0.848
	3	1	0.053	0.053	0.314	0.593
	4	1	0.049	0.049	0.922	0.369
	5	1	0.09	0.09	0.399	0.548
	6	1	0.072	0.072	1.782	0.224
	7	1	0.014	0.014	2.463	0.161
	8	1	0.012	0.012	0.14	0.719
	9	1	0.056	0.056	0.005	0.948
	10	1	0.077	0.077	1.232	0.304
Error	Sprint 1	6	0.15	0.021		
	2	6	0.071	0.01		
	3	6	0.117	0.017		
	4	6	0.037	0.053		

Source of Variation	DF	SS	MS	F	P
5	6	0.016	0.023		
6	6	0.028	0.041		
7	6	0.039	0.056		
8	6	0.061	0.087		
9	6	0.085	0.012		
10	6	0.043	0.062		

APPENDIX D INFORMED CONSENT

Virginia Tech
Informed Consent for Participants of Investigative Projects
Department of Human Nutrition, Foods, and Exercise
Title: The Effect of Creatine Supplementation on Muscle Energy Stores, Body Composition, and Exercise Performance During Energy Restriction
Principal Investigators: John Rockwell and Janet Walberg Rankin Ph.D., faculty advisor.
Purpose: Some athletes take creatine supplements with the thought that this will increase muscle stores of creatine and creatine phosphate, important fuels for high intensity exercise. There is some evidence that these supplements may also increase lean, muscle tissue. Some studies on rats and obese individuals that have undergone energy restriction show that muscle energy stores may decrease. This study will determine whether a short term low calorie diet will reduce muscle energy stores in active weight lifters and whether supplementary creatine will change muscle fuel stores, influence body composition change or high intensity exercise performance during this weight loss. This has implications for athletes who are trying to lose weight but want to maintain performance and muscle mass.

Procedures: In the 30 days leading up to the study period, it is important that you refrain from supplementing creatine. This allows for any creatine you've already supplemented to clear from your body and allow muscle creatine levels to return to normal. Two weeks before the study period, you will be weighed on a medical scale. You will be weighed again the day before energy restriction begins, to determine if your weight has remained stable for two weeks. We want you to be weight stable during the time prior to the study. So, if your weight changes more than 1 pound or so after the two week period, you will not be included in the study. You will be weighed each day of the study period.

The actual study will last 9 days. On days 1 and 3, you will perform two practice performance tests on the exercise cycle, each followed by a day of rest. The test will consist of ten 6 second maximal cycling sprints, with 30 seconds recovery after each sprint. It is important that you give a maximal effort with each test.

On day 5, you will be assessed for body composition using the hydrostatic weighing tank. You will sit in a chair immersed in water, and once the air in your lungs is completely expelled, measurements of your body weight underwater will be taken. The residual volume of your lungs (the amount of air left at the end of a maximal expiration) will be measured by an oxygen rebreathing technique, in which you will breathe five times into a rubber bag filled with oxygen while seated outside the tank. The underwater weighing and the lung volume measurement will take about 45 minutes.

A needle muscle biopsy will be taken from your thigh muscle. This will involve shaving part of your thigh, cleaning it, injecting a local anesthetic (feels like a bee sting), and making about a 1/2 inch incision with a scalpel once the area is numb. A hollow needle will be inserted through the incision to remove a small piece of your muscle (less than half the size of a pencil eraser). You will feel some cramping and discomfort but should not feel strong pain. When the needle is removed, we will put pressure and cold on the incision for about 20 minutes. The incision will be closed with steri-strips (similar to Band-Aids) and will be covered with a pressure wrap. The pressure bandage should be

left on for about 8 hours. The steri-strips should remain on for about 3 days. You should not "baby" the leg; using it will prevent excessive stiffness. You may take over the counter pain medication if you feel that is necessary. We will be giving you instructions on care of the incision, warning signs to watch for and phone numbers to contact should you have any questions. The incision will close and begin healing within a few days but a small scar will remain. The muscle biopsy sample we remove will be frozen and later analyzed for muscle fuel content. The biopsy procedure lasts about 30 minutes. Following the biopsy, you will do performance test 1 (identical to the practice tests). The performance test along with the warm up and cool down will take about 15 minutes.

Day 5 (following the above tests) through day 8 will be a time of weight loss through energy restriction for the experimental groups. Those subjects in the control group will eat normally throughout this period and will be asked to maintain your weight. Subjects in the weight loss groups will be given supplements (this may be creatine (Cr) or a placebo (P), sugar) to take 4 times per day on days 5-8. During the energy restriction period, you will consume a formula diet consisting of cans of a liquid meal replacement (60% carbohydrate, 20% protein, 20% fat, Ensure, Ross Laboratories). You will receive 18 kcal of Ensure for every kg (1 kg is 2.2. pounds) of your body weight (e.g. 1350 kcal for a 165 pound person) each day. It is very important that no other foods be consumed during this time, although noncaloric beverages may be consumed. Our experience in the past suggests you will probably lose from 2 to 6 pounds during this period.

On day 9, you will again have your body composition assessed using the hydrostatic weighing tank and the same procedure as before. Body composition analysis will be followed by another muscle biopsy taken from the same thigh muscle as described above.

Timeline:

day 1 - Practice performance test
day 2 - Off
day 3 - Repeat practice performance test
day 4 - Off
day 5 - Body composition analysis. Performance test 1. Needle muscle biopsy to assess muscle Cr and CrP levels; begin energy restriction, supplementation and urine collection.
day 6 - Energy restriction. Cr or P supplements 5g, 4 x per day
day 7 - Energy restriction. Cr or P supplements 5g, 4x per day
day 8 - Energy restriction. Cr or P supplements 5g, 4x per day
day 9 - a.m.: Body composition test. Performance test 2. Needle muscle biopsy.

Subject Responsibilities:

1. Attendance at a meeting to assess qualification to participate in the study.
2. Refrain from supplementation of creatine for 30 days prior to the start of the energy restriction period.
3. Subjection to measurements of body weight two weeks prior to energy restriction, body composition analysis by hydrostatic weighing, high-intensity cycling performance

4 times (2 practice, 2 tests), and needle muscle biopsy before and after the energy restriction period.
4. Ingestion of 5g creatine, 4 times per day during energy restriction.
5. Consume only the formula diet provided by the researchers, with no other source of calories.
6. Give maximum efforts on the performance tests.
7. Attend exercise and body composition testing in the Human Performance Laboratory in War Memorial Gym.
8. Inform the researchers of any medical conditions or injuries that may arise during the study or affect the results, or of any known transmittable diseases such as HIV or hepatitis.
9. Inform the researchers of any history of steroid use.
10. You must remain in the laboratory for at least 20 minutes following the muscle biopsy and must return to the laboratory for us to look at the incision for two days following each biopsy.
11. You must inform the experimenters of any problems associated with the site of muscle biopsies (e.g. soreness, infection).

Risks of Participation:

1. Hunger, irritability, constipation, and fatigue are possible during the energy restriction diet. These conditions will be alleviated after you begin eating a normal diet after the low calorie phase.
2. Fatigue, muscle soreness, and muscle strains or pulls may result from the performance test. Since you are an experienced resistance trainer, the performance test is unlikely to cause any muscle problems.
3. Infection, bruising, muscle soreness from needle muscle biopsy. The procedures will be conducted by an experienced technician licensed to perform these tests. Universal precautions will be taken such as use of gloves when handling tissue samples. Your blood will be screened for HIV if there is exposure of your blood or muscle to any experimenter (Any person who is exposed will also have their blood tested for HIV immediately upon exposure to provide a baseline value. Thus, if they already have HIV prior to exposure to your blood, any subsequent infection will not be attributable to this exposure).
4. The incision from the muscle biopsy may be tender for a few days after the biopsy. Infection is possible, this risk is reduced by keeping the incision clean. We will look at the incision for two days following the biopsy to insure that it is healing appropriately. You will have two small scars on the thigh of your leg. Like any other scar, they will be red at first and fade with time. Note that the University will not be responsible for any medical expenses you may have unless the University has been negligent. We have not had anyone who required any medical attention following biopsies in the past.
5. An allergic reaction is possible to the local anesthetic used during the muscle biopsy. It is important that you tell us if you know of any allergies to medications or have ever experienced a reaction to Novocain at the dentist office.

- A telephone will be available at all testing sites to notify emergency services (Virginia Tech rescue squad) of any medical emergencies. Only Janet Rinehart, certified medical laboratory technician will perform the biopsies. A physician will be available in case of any emergency. The investigators involved in the performance test will be trained in basic first aid and CPR.

Benefits of Participation

Your participation will provide you with:

1. Data on your body composition.
2. A total compensation of $40 for the 16 experimental subjects, and $10 for the 8 control subjects will be given upon completion of the study. If you drop out or are asked to leave the study prior to completion, you will receive $2 per day for days 1-4 and $5 per day for days 5-9 of the experiment.

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 consent. Data will be identified by subject numbers, without anything to identify subjects by name. Only the researchers will know your identity.

Freedom of Withdrawal

You are free to withdraw at any time from this study for any reason without penalty. Circumstance in which the investigator may determine that you should not continue as a subject in the study include but are not limited to: lack of compliance to the prescribed diet, failure to attend measurement sessions, and illness.

Approval of Research:

This research project 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.

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: John Rockwell, Master's Candidate, Dept of Human Nutrition, Foods and Exercise (540) 961-5481
- Project Chairperson: Dr. Janet Walberg Rankin, Ph.D. Dept of Human Nutrition, Foods and Exercise (540) 231-6355
- Chairman - Institutional Review Board/Research Division: Tom Hurd (540) 231-5281

Name of Subject (please print) _____

Signature of Subject: _____

Date: _____

APPENDIX E INSTITUTION REVIEW BOARD PROPOSAL

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

TITLE: The Effect of Creatine Supplementation on Muscle Fuel Stores, Body Composition, and Exercise Performance During Energy Restriction

INVESTIGATORS: John Rockwell
Janet Walberg Rankin, Ph.D., advisor

JUSTIFICATION:

One purpose of this investigation is to determine whether the creatine phosphate stores (the muscle fuel used for high intensity muscular efforts) of weightlifters are reduced during energy restriction. Secondly, we will determine whether creatine supplementation during energy restriction will affect muscle stores of creatine phosphate, physical performance, and body composition changes.

Athletes in several sports attempt to maximize percentage of lean body mass by energy restriction to lose fat mass, in anticipation of improved exercise performance or attaining a weight class. Severe energy restriction in preparation for competition has been widely reported in wrestlers, weightlifters and bodybuilders. One drawback to such an approach to controlling body composition is that while fat mass is decreased during energy restriction, muscle mass is decreased as well.

Supplementation of creatine monohydrate is a popular practice among athletes wishing to improve high-intensity exercise performance. Many athletes at Virginia Tech are using creatine, often at the suggestion of coaches and strength trainers. Several types of supplements are available, from creatine powder and powdered drinks to creatine energy bars. Currently, the available literature is divided as to the benefits of creatine supplementation.

Creatine phosphate is the first fuel utilized for energy during high-intensity exercise. Muscle creatine stores are increased and hopefully maximized as a result of creatine supplementation of 20 to 25 grams per day. In repeated high-intensity exercise bouts, these additional creatine stores allow for quicker regeneration of creatine phosphate and therefore quicker recovery.

Studies using obese humans and rats have shown that energy restriction results in decreased muscle creatine levels. It is also known that energy restriction can impair performance due to reduced energy stores and dehydration. Thus, it is reasonable to hypothesize that supplementation during energy restriction will help maintain muscle creatine stores and exercise performance.

PROCEDURE:

Twenty four male resistance trainers age 18 to 25 will be selected. Subjects will have been training for at least 2 years, 3 to 6 times per week, with no reported history of anabolic steroid use. Fliers will be posted in weight rooms on campus and in town announcing the study. The fliers will include a list of measurements that will be made (including muscle biopsies) as well as the statement that individuals taking anabolic steroids will not be used in the study. Interested individuals will call the experimenters to

get further brief information about the study. Those who remain interested will be invited to attend a group session where the study will be explained in detail. Those who are still interested will be given informed consent forms. Those returning the signed consent will fill out detailed screening forms regarding their health history and exercise habits. Volunteers will be screened for contraindications to weight loss and resistance exercise such as diabetes, heart conditions, orthopedic limitations and injuries, epilepsy or seizures, hypoglycemia, mental illness, pulmonary disease, and major organ malfunctions. Those subjects
who report phobia to water immersion or allergy to Novocain or iodine on the screening form will be excluded. Subjects will be chosen from the pool of low risk subjects by the experimenters. Subjects who have previously supplemented with creatine must abstain for 30 days to allow muscle creatine levels to return to normal levels prior to the start of the study.

Subjects will be randomly assigned to three groups: placebo, creatine supplementation, or control. A high-intensity, repeated bout performance test will be done before and after a four day energy restriction period. Creatine supplementing subjects will consume 5 g of creatine monohydrate 4 times a day during the energy restriction period while placebo subjects will consume a supplement of similar appearance (lactose). Muscle biopsies will be performed on the placebo and creatine supplement groups before and after energy restriction to establish the effects of energy restriction and creatine supplementation on muscle creatine levels. Total daily urine collections will be done for each day of the energy restriction, formula diet phase. Control subjects will participate in the performance and body composition tests but will not undergo energy restriction, supplementation, muscle biopsies or urine collection.

Diet and Supplement: The control group will eat a normal unrestricted diet throughout the experiment. Placebo and creatine supplement groups will consume a formula energy restriction diet of 18 kcal/kg/day. The hypoenergy diet will consist entirely of Ensure, Ross Laboratories, which is 60% carbohydrate, 20% protein, and 20% fat. No other source of calories will be consumed by the subjects. We have used the same diet in wrestlers for 3 d and a similar formula diet in weight lifters for up to 10 d. Creatine supplementing subjects will consume 5g of Cr monohydrate, 4 times a day, while placebo subjects will consume 5g of placebo, 4 times a day, throughout the energy restriction period.

Body Weight: Body weight maintenance prior to the start of the energy restriction period will be determined by weighing subjects during the 2 weeks prior to the experimental period. Anyone with more than a 0.5 kg change over the 2 weeks will be excluded. Body weight will be assessed to the nearest 0.1 kg on a medical scale each day of the experimental period.

Body Composition: Hydrostatic weighing of placebo and creatine supplement groups before and at the end of energy restriction will determine the effects of energy restriction and creatine supplementation on body composition. During this test, the subjects will have to submerge, blow as much air out of their lungs as possible and stay still for 2 seconds while a body weight measurement is taken. A load cell attached to a chair submerged in a hydrostatic weighing tank (Novel Products, Rockton, IL) sends the information on subject weight to a computer. The three highest values obtained from

eight measurements will be used. Residual volume of the lungs will be measured by the oxygen dilution technique, where seated subjects breathe five breaths into a oxygen filled rubber bag. The oxygen and carbon dioxide content of this bag is determined and used to calculate lung residual volume.

Performance Test: The exercise performance test will consist of ten repeated leg sprints on a exercise bike. Bouts of 6 seconds each of maximal effort cycling will be separated by 30 seconds of passive recovery. Two practice tests (day 1 and 3) will be performed separated by 48 hours prior to the experimental period to reduce the learning effect. The performance test will occur prior to energy restriction on day 5 and after energy restriction on day 9. Reliability of the performance test will be assessed using data from the tests on days 3 and 5. The test will be performed on a cycle ergometer, and total work performed and peak power will be measured.

Muscle Biopsies: A portion of the vastus lateralis (thigh) muscle will be removed using the needle muscle biopsy technique with suction to determine muscle free creatine and creatine phosphate content. The procedure will be performed prior to and at the end of energy restriction (on days 5 and 9) by Ms. Janet Rinehart (certified medical laboratory technician) with a physician available. 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 25-75 mg sample of muscle is removed using a hollow needle that is inserted through the muscle fascia. The incision is closed 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 are provided with phone numbers to contact the experimenters or observing physician should they have any questions. The muscle sample will be frozen immediately in liquid nitrogen, freeze-dried, powdered, and stored at -80 degrees. The samples will be analyzed for creatine and creatine phosphate using enzymatic spectrophotometric techniques.

Urine samples: Subjects will be asked to collect all their urine for the four days of the formula, hypoenergy diet. They will be provided each day with jugs containing a small amount of preservative for this purpose. We will be analyzing the urine for total nitrogen and creatinine. This provides information concerning loss of body protein during the weight loss.

Timeline:

day 1 - Practice performance test
day 2 - Off
day 3 - Repeat practice performance test
day 4 - Off
day 5 - Body composition analysis, Performance Test 2, Needle muscle biopsy.
day 6 - Begin energy restriction for P and CrS groups. Cr supplements 5g, 4 x per day, urine collection
day 7 - Energy restriction. Cr supplements 5g, 4x per day, urine collection
day 8 - Energy restriction. Cr supplements 5g, 4x per day, urine collection
day 9 - a.m.: Body composition test, Performance test 2, Needle muscle biopsy.

RISKS AND BENEFITS:

Risks associated with this protocol include hunger, irritability, and fatigue with the energy restriction diet. Fatigue, muscle soreness, and muscle strains or pulls may result from the performance test. Creatine supplementation has not been reported to have any harmful side effects. The subjects will be resistance trainers familiar with exercise equipment, and will perform two trial performance tests before the actual performance test. Risks of the muscle biopsy include allergic reaction to the local anesthetic. We will reduce the chance of this by requesting information concerning allergies to Novocain (similar effects to Lidocaine) at the dentist office. The subjects will be verbally asked this question again just prior to the biopsy procedure. The biopsy will result in a small scar and could become infected. Subjects will be educated on the care of this incision to avoid infection. We will also require the subjects to return to the laboratory for two days following each biopsy so that we can examine the area. Only Ms. Janet Rinehart, certified medical laboratory technician in our department, will be performing the biopsies. She was trained at East Carolina University in this procedure and has completed over 90 biopsies at Virginia Tech without negative consequences. We will have a physician available at the time of the biopsies in order to handle any emergencies should they arise. Any medical expenses of the subjects will not be covered by the University unless the University is negligent. Benefits from this study include data on body composition and compensation of $40 for the 16 experimental subjects, and $10 for the 8 control subjects.

CONFIDENTIALITY:

The data from this study will be kept strictly confidential. No data will be released to anyone but the principal investigator and the advisor without the subject's written consent. Data will be identified by subject numbers.

BIOGRAPHICAL SKETCH:

John Rockwell, MS candidate: Graduate Student in Nutrition for Sports and Chronic Disease option in the Human Nutrition, Foods, and Exercise department at Virginia Tech. He earned a bachelor's degree in Kinesiology with a minor in Biology at the College of William and Mary. He helped with another research study this spring that involved diet and exercise manipulation with human subjects and the collection of blood samples.

Janet Walberg Rankin, Ph.D., Faculty Advisor: Dr. Rankin earned a bachelor's degree in zoology from Duke University and a doctorate in Nutrition with a minor in Exercise Physiology from the University of California at Davis. She has been on the faculty at Virginia Tech since 1982 and is currently an Associate Professor in the Department of Human Nutrition, Foods and exercise. She teaches undergraduate classes in "Exercise Physiology" and "Nutrition and Physical Performance" as well as a graduate class in "Metabolic Aspects of Exercise." Her primary research areas are nutritional manipulations in athletes and weight control. Her research has been published in journals such as: International Journal of Sports Nutrition, Medicine and Science in Sports and Exercise, and International Journal of Sports Medicine.

APPENDIX F INSTRUCTION FOR SUBJECTS

On testing days, Monday and Friday, bring appropriate clothing and shoes to wear during a sprint cycle test, as well as a towel and bathing suit for the underwater weighing tank. You will be weighed and given food and supplement for the day each morning at 8am in room 230 War Memorial Hall. At this time we will give you urine collection bottles for the day, and collect urine collection bottles from the previous day. We will look at your supplement log each morning. For the muscle biopsies, bring a pair of shorts. Muscle biopsies will be performed in Cassell Coliseum, room 118.

Low Calorie Diet

The purpose of the formula diet is to control Calorie as well as nutrient intake. The diet will supply 18 Calories per kg body weight (1 kg is 2.2 pounds), so a 165 pound person will consume 1,350 Calories per day. This is not a starvation diet. Food will be supplied to you each day in the form of Ensure, a nutritional supplement that is 55% carbohydrate, 21% protein and 24% fat. The diet will provide all of the recommended amounts of vitamins and minerals, so vitamin supplementation is not necessary.

You can expect to lose about 1-3 pounds of body fat, and even more water weight. You are likely to regain the weight once the experiment is over. Drink plenty of water, at least six cups per day or more. Since weight loss can result in dehydration, you may feel tired and dizzy. Consuming enough water will prevent this from occurring.

It is very important that you consume all of the Ensure that you are given each day, and that you do not consume any other Calories.

It is OK to consume non-Caloric products while on the diet, such as:

- Water
- Diet soft drinks
- Coffee (artificial sweeteners such as Equal and Sweet-n-Low only)
- Tea (artificial sweeteners only)
- sugarless gum

If you think there is something else that could be OK to eat during the diet, check with us first. It is also OK to heat the Ensure in the microwave, or blend it with ice. It tastes best cold, so it is suggested you store it in the refrigerator.

Expect to feel some fatigue on the diet, and you may also experience a change in your bowel habits. This is normal. ***Let us know if you experience any unusual symptoms.***

The Low Calorie Diet begins on Monday. You should not eat anything before performing the bike test and hydrostatic weighing test that morning. You will be given 2 cans of Ensure to consume after the bike test and before the muscle biopsy.

Urine Collection

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

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

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 room 230 War Memorial Hall each morning when you pick up your diet and supplement. You will then be given new bottles for the next 24 hour collection period.

Supplement

Supplementation will occur for four days, including ½ day Monday, after the muscle biopsy, and ½ day Friday, before the muscle biopsy.

On Monday, you will be given two supplement packets, to be taken after the biopsy procedure, and on Friday you will be given two packets to be taken before the biopsy procedure.

On Tuesday, Wednesday and Thursday you will be given four supplement packets, to be taken in the morning, noontime, afternoon, and evening. Write the time that you take your supplement/Ensure on the supplement log. Keep the log with your Ensure and supplement packets at all times.

When taking the supplement, mix the entire supplement packet into one full can of Ensure, stir well, and consume the mix. Do not drink caffeinated beverages along with the supplement/Ensure, because caffeine can affect absorption.

Call me anytime with questions or problems	961-5481
If I'm not there, try the lab in WMH 230	231-8299
Or the Grad office in Wallace Hall	231-7708

Supplement Log

Record the times that you take the supplement/Ensure mix here, and show this to the investigators each morning.

Monday Afternoon

Time 1st supplement/Ensure was taken _____ (after biopsy, before 4pm)

Time 2nd supplement/Ensure was taken _____ (after 7pm, before bed)

Tuesday

Time 1st supplement/Ensure was taken _____ (breakfast time, when you wake up)

Time 2nd supplement/Ensure was taken _____ (lunchtime, around noon)

Time 3rd supplement/Ensure was taken _____ (afternoon, between 2 and 5pm)

Time 4th supplement/Ensure was taken _____ (after 7 pm, before bed)

Wednesday

Time 1st supplement/Ensure was taken _____ (breakfast time, when you wake up)

Time 2nd supplement/Ensure was taken _____ (lunchtime, around noon)

Time 3rd supplement/Ensure was taken _____ (afternoon, between 2 and 5pm)

Time 4th supplement/Ensure was taken _____ (after 7pm, before bed)

Thursday

Time 1st supplement/Ensure was taken _____ (breakfast time, when you wake up)

Time 2nd supplement/Ensure was taken _____ (lunchtime, around noon)

Time 3rd supplement/Ensure was taken _____ (afternoon, between 2 and 5pm)

Time 4th supplement/Ensure was taken _____ (after 7pm, before bed)

Friday Morning

Time 1st supplement/Ensure was taken _____ (after bike test, underwater weigh)

Time 2nd supplement/Ensure was taken _____ (lunchtime, before noon)

APPENDIX G EXIT QUESTIONNAIRE

This will not affect your subject payment in any way

The Effect of Creatine Supplementation on Muscle Creatine Stores, Body Composition and Exercise Performance During Energy Restriction

Exit Questions

Name _____ SS# _____ Date _____
(For Payment Purposes)

Address _____
_____

1. Do you think you know whether you received the Creatine or the Placebo supplement?

2. If so, which one do you think you were taking and why do you think so?

3. Were there any occasions where you didn't fully comply with the protocol in terms of urine collection, diet restriction, or supplement taking?

4. Was there anything that the investigators could have done to make your experience as a subject in the study better?

5. Were all of the instructions clear? Did you have any problems complying or feel that the investigators didn't communicate enough with you?

6. If our lab were to perform a future study, would you consider being a subject if the topic were of interest to you?

Thanks for your participation in this study

RESULTS

Question 1 Knowledge of supplement.

10 Yes. 4 No. 2 Maybe.

Question 2 What did you think you received?

6 correct (4 Cr, 2 Pl). 7 incorrect (3 Cr, 4 Pl). 3 no guess (1 Cr, 2 Pl).

Question 3 Did you fully comply with the experiment?

12 full compliance. 4 reported instances of non-compliance.

1 subject missed 1 supplement (Pl, lost the packet).
1 subject drank 2 sips of a soft drink (Cr).
1 subject missed 1 or 2 urine voidings (Cr).
1 did not specify (Cr).

Question 4 Could the study have been better?

12 No. 4 just comments.

Comments/Suggestions
-More $, of course!
-It was great
-It ran smoothly
-Why did we have to go on a low-calorie diet, what did that help?
-More Ensure
-Slightly higher-calorie diet. A group dinner with selected foods.
-No males rubbing males' legs!
-Better flavors of Ensure

Question 5 Were the instructions clear? Any problems with communication with investigators?

16 All clear. 16 No communication problems

Question 6 Would you be interested in a future study if the topic interested you?

11 Yes. 5 Maybe/Probably/Perhaps.

1 Not if there are biopsies.

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Vita

John Albert Rockwell was born on September 3, 1974 in Olney, MD. He graduated from the College of William and Mary in 1996 with a Bachelor of Science, majoring in Kinesiology and minoring in Biology. He was a four year varsity letterman on the swim team at William and Mary and team captain his senior year. He was named to the CAA all-academic team his junior and senior year. He was a member of the Sigma Chi fraternity and served as chapter president his senior year. He began graduate study at the Virginia Polytechnic Institute and State University in the fall of 1996, in the Department of Human Nutrition, Foods, and Exercise. His concentration was in Sports Nutrition and Chronic Disease.