CHAPTER I INTRODUCTION

Since the discovery of creatine in 1832, it has fascinated scientists with its crucial role in skeletal muscle metabolism. In humans, over 95% of the total creatine content (TCr) is located in skeletal muscle, of which approximately a third is in its free form. The remainder is present in a phosphorylated form (PCr). Both forms of creatine are present at levels in skeletal muscle which are subject to individual variations and influenced by factors such as muscle fiber type, age, disease, and inconclusively by other factors such as training or gender. A naturally occurring substance, creatine is found exogenously in foods such as meat and fish, while the remainder is derived endogenously by the liver, kidneys, and pancreas.

Creatine is replenished at a rate of approximately 2 g per day following irreversible degradation to creatinine. It has long been accepted that ingestion of creatine will add to the body's creatine pool. A century ago, studies with creatine feeding concluded that some of the ingested creatine was retained in the body (Balsom et al., 1994). Since that time, researchers have been investigating the potential benefits of creatine with respect to skeletal muscle performance.

Maximal intensity exercise of short duration relies almost exclusively on the creatine phosphate system whereby PCr is degraded anaerobically. It has been suggested that a decline in force production may be related to depleted PCr and Cr stores which may limit the rate of ADP rephosphorylation. Thus, it has been hypothesized that increasing the total available PCr stores may reduce the rate of PCr depletion during maximal contraction and aid the rate of ADP rephosphorylation. Subsequent studies have suggested that TCr levels in skeletal muscle may enhance performance of high-intensity exercise following a period of creatine supplementation (Greenhaff, 1995).

Two mechanisms may explain the importance of creatine in the enhancement of exercise. Primarily, creatine supplementation may increase PCr stores in the muscle and thus delay depletion. The increase in storage of creatine may increase the rate of PCr resynthesis leading to higher productivity in training, and in turn, maximal power (Balsom et al., 1994).

There may be an interaction between exercise training and creatine supplemention. Harris et al. reported an enhancement of the increase in TCr in subjects who consumed a creatine supplement concurrent with training. The increase in TCr was more profound in the exercised skeletal muscle, suggesting that training may influence the effect of dietary creatine on muscle PCr.

Creatine studies have produced conflicting evidence regarding its efficiency with respect to performance. A few studies have reported performance enhancement with creatine supplementation (Greenhaff et al., 1993, Balsom et al., 1993, Harris et al., 1993), while a similar number of studies show no benefits (Cooke et al., 1995, Terrillion et al., 1996, Ruden et al., 1996).

STATEMENT OF THE PROBLEM

Few studies to date (the majority of which are published only as abstracts) have examined the effects of chronic creatine supplementation during resistance training. Five male subjects increased their absolute 1RM on bench press and their total lifting volume after

supplementation (Earnest et al., 1995). Unlike previous research on creatine in which subjects were supplemented 5-7 days, Earnest et al., supplemented for 28 days. In addition, studies which have investigated strength training and creatine did not include body composition variables.

The majority of studies examining creatine supplementation have used only male subjects. A few studies have combined male and female subjects but did not report effects seperately by sex. The studies available which relate to creatine content and gender are also inconclusive. One study demonstrated that females have a higher TCr store in relation to tissue weight (Forsberg et al., 1991). Overall, there is a lack of information regarding the effects of creatine on women. Creatine content was seen to increase most dramatically in those consuming a vegetarian diet (Delanghe et al., 1989). It is likely that a population who are historically more conscious of their body composition and thus undergo frequent energy restriction may benefit greatly from creatine supplementation. Creatine may not be sufficiently supplied by their non-carnivorous and/or low calorie diets. This idea makes female subjects attractive candidates for a creatine supplementation study.

The purpose of this study was to examine the possible effects on performance of chronic creatine supplementation on females participating in resistance training. The independent measure was the creatine supplementation and strength training protocol, while the dependent measures were 1RM bench press, 1 RM leg extension, and muscular endurance (isokinetic knee extension, 5 sets of 30 repetitions at 180 degrees/sec.) tests to assess the potential performance enhancement of this new ergogenic aid. Additional measures such as lactate response to the isokinetic knee test, body weight, percent body fat by skinfold and hydrostatic techniques, fat-free mass by skinfold and hydrostatic techniques, body water as assessed by bioelectric impedence, and blood metabolites (BUN and GPT to assess kidney and liver function) were investigated.

SIGNIFICANCE OF THE STUDY

Previous research has suggested that creatine supplementation may or may not improve anaerobic exercise performance in males. It is not clear whether there is also a benefit of this supplement for females. Most studies have been performed using short-term supplementation, but there is some evidence that there may be some synergistic effect of long-term supplementation with concurrent strength training (Earnest et al., 1995).

As no previous studies have used female subjects exclusively, this study may be beneficial to other females interested in improving performance. Furthermore, very few studies have investigated creatine supplementation with strength training, and little conclusive evidence is available regarding creatine supplementation on any performance parameter.

RESEARCH HYPOTHESIS

The following hypotheses originated from the development of this study:

Ho: There was no significant difference in 1RM bench press strength, work fatigue or total work as measured by the isokinetic knee extension test, or 1 RM leg extension strength in women after 5 weeks of creatine supplementation combined with strength training 3 times per week.

Ho: There was no significant difference in body water, body weight, or percent bodyfat between the placebo and creatine groups.

Ho : There was no significant difference in blood lactate accumulation, GPT, or BUN between the placebo and creatine groups.

DELIMITATIONS

In this study, the following delimitations were imposed by the researcher:

1. Subjects were limited to apparently healthy 18 to 22 year-old female lacrosse players at Virginia Tech who had no health problems as defined by a health questionnaire.
2. The creatine supplementation period involved administration of 20 g per day of creatine monohydrate for 7 days, followed by 3g per day for 32 days.
3. The dependent measures were limited to achievement in 1RM bench press, 1 RM leg extension, and an isokinetic knee extension endurance test performed before and after the 5 week period.

LIMITATIONS

The following limitations affect any generalizations which evolved from this research:

1. The results of this study only apply to this sample of college-aged female lacrosse players selected on the basis of adequate health to participate in this study.
2. The results are limited to this particular dosage regimen of creatine monohydrate.
3. The results are limited to the strength training program used in this study.
4. The diet habits if the subjects were not monitored.

BASIC ASSUMPTIONS

The following assumptions were made by the investigator:

1. The efforts exerted by the subjects for the 1RM tests andthe knee extension tests were maximal.
2. The subjects' diets and activity levels were kept constant throughout the study.

DEFINITIONS AND SYMBOLS

Total creatine (TCr): phosphocreatine + free creatine

Phosphocreatine/Creatine phosphate (PCr) : phosphorylated form of creatine making up 2/3 of total creatine

Creatine monohydrate (Cr.H20): commercial supplement found in capsule or powder form

One repetition maximum (1RM): amount of weight in lbs. which can be lifted once according to specified criteria

Isokinetic knee extension: measurement of quadricep power at a fixed speed and variable resistance

Bioelectric impedance analysis (BIA): measurement of body water using small electrical current through the body

Glutamyltransferase (GPT): liver enzyme monitored to evaluate kidney function

Blood urea nitrogen (BUN) : blood metabolite monitored to evaluate kidney function

SUMMARY

Skeletal muscle contraction and relaxation is fuelled by the energy released upon dephosphorylation of ATP. Maximal intensity exercise of short duration relies almost exclusively on the creatine phosphate system whereby PCr is degraded anaerobically to release energy for rephosphorylation of ADP. It has been suggested that a decline in force production may be related to depleted PCr stores which may limit the rate of ADP rephosphorylation (Balsom et al., 1994). Thus, it is possible that increasing the total available PCr and Cr stores may reduce the rate of PCr depletion during maximal contraction and aid the rate of ADP rephosphorylation, translating into potentially increased maximal performance.

Lately, much attention has been devoted to the use of creatine in order to enhance performance. Neither endurance exercise nor maximal oxygen uptake appears to be enhanced, nor have any adverse side effects of creatine supplementation been reported. A few studies have demonstrated increased maximal power output with creatine supplementation (Greenhaff et al., 1993, Balsom et al., 1993, Harris et al., 1993), while anequal number of studies have reported no impact of creatine supplementation on performance (Cooke et al., 1995, Terrillion et al., 1996, Ruden et al., 1996). Few published studies have examined the effects of creatine supplementation on strength-trained subjects (Earnest et al., 1995), and even fewer studies have investigated if females may benefit from creatine supplementation. Discovering if chronic creatine supplementation can improve performance in strength-trained females may add to the debate regarding creatine as an ergogenic aid.

CHAPTER II REVIEW OF LITERATURE

INTRODUCTION

Since the discovery of creatine in 1832, scientists have been facinated with its central role in skeletal muscle metabolism. As a result of creatine's potential ability to improve several performance parameters, it has become a popular research topic in the sports nutrition world. However, creatine studies have produced conflicting evidence regarding its efficiency with respect to performance.

HISTORICAL BACKGROUND

In 1832, Chevreul, a French scientist, reported the finding of a new organic constituent of meat to which he gave the name 'creatine'. Due to the problems with the method for detecting creatine, it was not until 1847 that Lieberg observed that the flesh of wild foxes killed in the chase contained 10 times as much creatine than did their captive counterparts. He concluded that muscle work involves an accumulation of creatine, and that creatine was a regular constituent of mammalian flesh (Balsom et al. 1994). Around this time, two other researchers, Heintz and Pettenkofer discovered a substance in urine, later confirmed to be creatinine. Following the observation that creatinine excretion was related to muscle mass, it was speculated that the creatinine found in urine was derived directly from the creatine stored in muscles (Greenhaff, 1995).

Further studies regarding creatine continued early into the twentieth century. Attempts were made to alter body creatine with dietary creatine. Creatine feeding required creatine extraction from fresh meat (a costly process), or less productively from urine. It was observed that not all creatine ingested by both animals and humans was excreted, which suggested that some of the creatine was retained in the body. The fate of this exogenous creatine was partly explained by a 70% increase in muscle creatine content in cats after creatine ingestion. By 1923 two researchers, Hahn and Meyer, had estimated the total creatine content of a 70 kg male to be approximately 140 g, which is close to the value predicted today (Volek et al. 1996).

In 1927, the discovery of Pcr in the resting muscle of cats led to concurrent demonstrations that during electrical stimulation of the muscle, Pcr diminished, only to reappear again during the subsequent recovery period (Hunter et al. 1928). Since this time, creatine in its free and phosphorylated forms has been recognized as a key intermediate of skeletal muscle metabolism.

Today's methods of creatine research have been improved by the reintroduction of the muscle biopsy technique. Hultman et al. first used this method in 1967 to study the breakdown and resynthesis of ATP with exercise in humans. Nuclear magnetic resonance spectroscopy has also been used more recently to investigate creatine's involvement in skeletal muscle metabolism (Pichard et al.1988).

CREATINE SYNTHESIS

Synthesis of creatine relies primarily on three amino acids: glycine, arginine, and methionine. The amidine group is transferred from arginine to glycine to form guanidinoacetate and ornithine. Transamidase is the enzyme reponsible for this reversible reaction, after which creatine is formed by the irreversible addition of a methyl group from S-adenosylmethionine (Balsom et al. 1994).

In humans, the liver, pancreas, and kidneys are sources of the enzymes responsible for creatine synthesis. The bloodstream carries 50 to 100 umol/L of creatine and delivers it to the muscle, where 95% of it can be found. The other 5% resides in the heart, brain, and testes. Creatine is also acquired exogenously by the ingestion of meat, fish, and some other animal products. The average intake of creatine from a balanced diet is approximately 1 g per day. The rate at which endogenous creatine is synthesized has been proposed to be dependent on a feedback mechanism (Volek et al. 1996). Thus, as in the case of vegetarians, daily requirements must be made endogenously. The mean creatine level in the human male quadriceps muscle is approximately 124.4 mmol/kg wet wt based on an enzymatic method on freeze dried muscle biopsy samples. (Delanghe et al. 1989)

CREATINE METABOLISM DURING MUSCLE CONTRACTION

Skeletal muscle contraction relies on ATP which is hydrolyzed to ADP. As energy demands increase, Pcr is degraded and the phosphate is donated to the ADP to regenerate ATP. The enzyme responsible for this reaction is creatine kinase.

Pcr is critical for ATP regeneration during high-intensity, single-bout exercise (Balsom et al. 1994). The total amount of muscle Pcr available initially to the muscle may influence the energy output and the onset of fatigue (Tesch et al. 1989). In humans, the importance of Pcr in maintaining power output during sprinting has been demonstrated by Hivronen et al. (1992) who observed that Pcr stores before and after exercise in the vastus lateralis were depleted by 63-71% in subjects 5 to 7 s following sprints of 40, 60, 80, and 100m. They concluded that the decrease in running speed that occurred after 5 s of exercise may be related to the decline in ATP production from the high-energy phosphate stores. The researchers found that in short-term maximal exercise (40-100m), the decrease in running speed began when the high-energy phosphate stores were depleted. In addition, the resynthesis of Pcr may also be a factor in performance as energy demands may be met for longer with a greater store of creatine in the muscle.

High-intensity exercise of short duration greatly increases the ATP demand of the muscle compared to rest. Electrical stimulation of a quadriceps muscle showed the rate of ATP turnover to be approximately 13 mmol/kg dm/s during a 10-s cycle sprint of two healthy male subjects (Jones et al.1985), and 15 mmol/kg dm/s during 6 s of maximal cycling in eight male subjects (Gaitanos et al.1993). Furthermore, the exercise Pcr concentration fell 57% from resting values in the Gaitanos study, and fell 50% from resting values in the Jones study. This research suggests that the creatine phosphate stores could be significantly reduced within 10 seconds.

A recent study by Bogdanis et al. (1995) has further demonstrated the importance of creatine phosphate for the recovery of muscle function following exhaustive, high-intensity exercise. Fourteen male subjects performed two 30 s cycle ergometer sprints separated by 1.5, 3, and 6 minute recoveries, on three separate occasions. On a fourth occasion, the subjects performed one 30 s sprint after which vastus lateralis biopsies were obtained. At the end of the 30 s sprint, Pcr contents were 19.7 $\pm$ 1.2 % of the resting values. During recovery, Pcr increased rapidly to 65.4 $\pm$ 2.8% of resting values after 1.5 minutes, and reached 85.5 $\pm$ 3.5% of rest after 6 min of recovery.

Soderland et al. (1991) examined the pattern of Pcr regeneration in different fiber types. They obtained biopsies from the vastus lateralis muscle of male and female adults at rest,

immediately after electrical stimulation with occluded circulation (total time 166 s), and after 20s, 60s, 5, and 15 minutes of recovery. The Pcr pool was depleted by 73% immediately after exercise. No significant rise in Pcr was noted after 20 s post-exercise. However, after 60 s, the Pcr content in type I fibers was 59% of the initial value compared to the type II fibers which had only regenerated 45% of the initial Pcr. However, after 5 minutes of oxidative recovery, the Pcr values were equal in the two fiber types, in addition to their resting values. Fifteen minutes post-exercise, type II fibers demonstrated a content of 97.8 mmol/kg dry wt, significantly higher than the resting value of 83.3 mmol/kg dry wt. Hultman et al. (1967) and Soderland et al. (1991) have studied the resynthesis of creatine phosphate and believe that total resynthesis is complete after 5 minutes. However, other researchers (Bogdanis et al. 1995) have suggested that only 85% of Pcr returns 6 minutes after 30 s of cycling.

The resynthesis of creatine phosphate during submaximal exercise has also been investigated (Hultman et al.1967). Creatine phosphate is not considered to be a primary energy substrate during low-intensity exercise; an inverse relationship has been reported between exercise intensity and creatine phosphate levels at the end of the exercise bout in the working muscles (Hultman et al. 1967). Although it appears that creatine phosphate levels decrease by 44% after 5 minutes of exercise between 60-70% of VO2 max and remain unchanged up to 20 min at the same intensity (Hultman et al.1967), muscle stores are not depleted to the same degree as during high-intensity exercise. (Balsom et al., 1994)

The reliance on muscle Pcr has also been investigated in resistance training. Tesch et al. (1986) examined the muscle metabolic changes occurring during intense, prolonged, heavy resistance exercise. Muscle biopsied were obtained from the vastus lateralis of nine strength-trained athletes before and 30 s after an exercise regimen comprising of 5 sets each of front squats, back squats, leg presses, and knee extensions using barbell or variable resistance machines. Each set was executed until muscle failure which occurred within 6-12 muscle contractions. The exercise:rest ratio was approximately 1:2 and the total performance time was 30 min. Concentrations of total creatine and Pcr were determined on freeze-dried samples using fluorometric assays. The total creatine value before exercise was 12.1 $\pm$ 3.4 mmol/kg wet and increased significantly to 23.8 $\pm$ 6.0 mmol/kg wet. Pcr values decreased significantly from 21.3 $\pm$ 3.7 mmol/kg wet before exercise to 10.9 $\pm$ 2.5 mmol/kg wet after exercise. This shows that the Pcr store was depleted by half at the end of the resistance exercises.

EFFECT OF GENDER, DIET, AND FIBER TYPE ON MUSCLE CREATINE

Few studies have compared creatine levels in skeletal muscle of males and females. Females have been found to have a higher concentration of total creatine in relation to tissue weight. Forty healthy males and females between the ages of 19 and 59 underwent the percutaneus needle-biopsy technique in order to investigate the human muscle content of total creatine (Forsberg et al.1991).

Previous research has suggested that creatine supplementation may improve anaerobic exercise performance in males. A few studies have used male combined with female subjects (Greenhaff et al.1993, Nevill et al.1989, Mujika et al.1996, Burke et al.1996) , but did not report effects separately by sex. Overall, there is a lack of information regarding the effects of creatine supplementation on women.

Chronic diet may also influence the levels of muscle creatine. Male and female vegetarians were found to have low levels of total creatine in serum compared to age- matched, non-vegetarians (Delanghe et al., 1989). Furthermore, creatine uptake was seen to increase most dramatically in subjects with a low initial total creatine content (Harris et al. 1992). Thus, vegetarians may get more increase in muscle creatine concentration through supplementation than meat eaters.

Fiber types have been suggested to contain different levels of creatine phosphate. Using freeze dried muscle biopsy samples, researchers have consistently found that type II human skeletal muscle has a higher level of creatine phosphate than type I fibers (Tesch et al.1989 and Essen et al. 1978). Tesch et al. obtained percutaneous muscle samples from the vastus lateralis of 12 active men at rest, immediately after an exercise bout consisting of 30 maximal voluntary knee extensions of constant angular velocity, and 60 s. Pcr content at rest was 82.7 and 73.1 mmol/kg dry wt in type II and type I fibers, respectively. After exercise the values were 25.4 and 29.7 mmol/kg dry wt. After 60 s of recovery, Pcr increased to 41.3 and 49.6 mmol/kg dry wt in type II and I fibers, respectively. It was concluded that basal Pcr content is higher in type II fibers, and it is also higher in type II fibers after a 60 s recovery period from short-term exercise (Tesch et al 1989). Edstrom et al. also found that the creatine phosphate level of the soleus muscle, which contains 65% type I fibers, was significantly lower than that of the vastus lateralis, which contains 41% type I fibers.

CREATINE AND EXERCISE TRAINING

Conflicting reports have been generated regarding the effect of training on creatine phosphate levels in human skeletal muscle. One study found a higher level of creatine phosphate in trained versus untrained individuals (Karlsson et al. 1971), whereas another failed to show such differences (Gariod et al. 1994). Gariod et al. compared the Pcr levels of children, adults age 20-35, 35-50, and 50 or more years with the Pcr content of volleyball players. Muscle biopsies were obtained on equal hemispherical tissue volumes (muscle plus skin and fat) corrected for thickness of skin and subcutanous fat. No differences in Pcr levels were noted among age groups, nor between athletes and non-athletes. Karlsson et al. (1971) investigated the Pcr levels of 14 military conscripts before, after 3, and 7 months of physical conditioning. At each testing time, needle biopsy samples were taken from each subject after performing three workloads. The physical conditioning consisted of 5-6 km of running as fast as possible 3 times per week, while the last 4 months consisted of the same runs only twice a week, in addition to cross-country skiing, swimming, or bicycling designed to enhance the subjects' aerobic power. The testing protocol involved two 10 min submaximal workloads: one at 150 W, and a higher level designed to demand 85% of VO2 max. During recovery before the training period, the mean Pcr level was 16.9 $\pm$ 0.8 wet wt, and increased after 3 weeks to 18.4 $\pm$ 0.5 wet wt, and finally returned to 16.8 $\pm$ 0.4 wet wt after 7 weeks. At the lower submaximal load (150 W), the Pcr concentration increased from 9.8 $\pm$ 0.8 wet wt during recovery before training, to 13.5 $\pm$ 0.5 wet wt after 3 weeks, and to 13.8 $\pm$ 0.5 wet wt after 7 weeks. Similar trends were noted in the Pcr levels during recovery at the higher submaximal workload. Finally, Bernus et al. (1994), using NMR spectroscopy, reported higher levels of creatine phosphate in the quadriceps muscles of sprinters compared to endurance runners. The researchers suggested a possible

explanation for the difference between athletic groups may have been a higher proportion of type II fibers in the sprinters.

Discrepancies in the effects of resistance training and/or high intensity exercise on muscle creatine have also been documented. Resistance weight training which induces muscle hypertrophy has been hypothesized to increase the absolute size of the total creatine pool. However, evidence from training studies which have used resistance training or high intensity exercise suggest that Pcr and Cr levels in skeletal muscle are not increased significantly from this type of training (Sharp et al.1986 and Nevill et al.1989). Sharp et al. (1986) had eight untrained males participate in 8 weeks of sprint training on bicycle ergometers. Muscle biopsy samples were taken from the vastus lateralis before and at 0, 5, and 15 mins following an incremental bicycle ergometer test. The analysis of biopsy samples showed no difference between pre- and post- training values of Pcr concentration. Nevill et al (1989) had sixteen males and females complete a maximal 30 s sprint and 2 minute run (designed to elicit 110% VO2 max) on a treadmill before and after eight weeks of sprint training. Muscle biopsies were taken from the vastus lateralis at rest and immediately after exercise. Total creatine was constant at a mean of 114 mmol/kg dry wt before and after exercise and before and after training for both groups. Both the 30 s sprint and the 110% run resulted in a 70% and 40% decrease of Pcr in both groups, respectively. No significant decrease in Pcr in the training group was found with either exercise test as a result of training compared to the control group. A later study was performed by Tesch et al.(1990) involving the effect of resistance training on total creatine and Pcr. Twenty-six active males were assigned to a concentric group, eccentric group, or control group. The subjects performed 5 sets of 12 eccentric or concentric maximal, voluntary bilateral quadricep contractions at 1.05 rad/s three times a week for 12 weeks. Bilateral percutaneous muscle biopsies were obtained from the vastus lateralis at rest pre- and post-training, and tissue samples were analyzed for total creatine and Pcr. Neither substrate was found to be significantly different in either group after 12 weeks of training. Others report that training with endurance exercise has failed to show changes in creatine levels (Karlsson et al 1972).

In summary, short-term training studies have failed to produce conclusive evidence regarding changes in skeletal muscle levels of creatine and creatine phosphate.

EFFECT OF CREATINE INGESTION ON MUSCLE CREATINE CONCENTRATION

Creatine is produced endogenously in the liver and pancreas at minimal amounts which are mainly stored in skeletal muscle. The average level of total creatine in human skeletal muscle was approximately 124.4 mmol/kg dry wt (Harris et al 1992), while the average Pcr concentration for humans before creatine supplementation was found to be 84.2 $\pm$ 7.3 mmol/kg dry wt. Investigations on creatine feeding in humans have confirmed earlier findings that the size of the body pool of creatine can be increased (Crim et al.1976). The average intake of creatine from a mixed diet is estimated to be 1g/day, however, the daily creatine requirement must be complemented by endogenous synthesis (Balsom et al.1994).

Creatine supplementation in humans is possible by oral administration of creatine monohydrate, (Cr.H2O), a white powder which is soluble in warm water. Creatine is transported into the muscle from the bloodstream, however, the exact mechanism by which it enters the skeletal muscle is unknown (Balsom et al., 1994).

Muscle creatine is replenished at a rate of approximately 2g/day, following its irreversible degradation to creatinine. During periods of dietary creatine supplementation, retention of creatine in muscle tissue is greatest during the initial stages of supplementation (Harris et al. 1992). In a recent study by Harris et al., with direct measurements from freeze dried muscle biopsy samples, the levels of skeletal muscle TCr were shown to increase in a group of 12 healthy male and female participants following a series of different regimens of creatine feeding. In this study, a 5g dose of Cr.H2O was administered four to six times a day for 4.5 days. The subjects varied greatly in their level of fitness and no restraints were placed on them in terms of diet or activity. A single muscle biopsy sample was taken from the vastus lateralis of each subject before and after the ingestion period. Significant mean increases in TCr were from 126.8 mmol/kg to 148.6 mmol/kg, while significant mean increases in Pcr were from 84.2 mmol/kg dry wt to 90.6 mmol/kg dry wt. The increases were subject to individual variances, and the uptake of creatine was related to initial TCr levels. Individuals with the lowest levels of initial creatine in their muscles appeared to achieve the most pronounced increases following supplementation. Thus, dietary supplementation increases total creatine, as well as Pcr, in the muscle.

Thompson et al. (1996) investigated the effects of chronic creatine supplementation by administering 2g/day for 6 weeks to collegiate swimmers in training. Magnetic resonance and spectroscopy were used to study the calf muscle metabolism in a group of female varsity swimmers at rest and during plantar flexion exercise. No differences were noted in muscle creatine concentration between the creatine and placebo groups.

Another study has documented the effects of varying dosages of creatine in 31 moderately-trained males (Hultman et al. 1996). Six subjects (group1) ingested 20 g creatine/day for 6 days, and muscle biopsies were taken from the vastus lateralis before supplementation, and on days 7, 21, and 35. Nine different subjects (group 2) ingested 20 g creatine/day for 6 days, and 2g/day for the next 28 days. Muscle biopsies were taken before, and 7, 21, and 35 days after supplementation. A third group of 9 subjects ingested creatine at 3g/day for 28 days. Muscle biopsies were obtained on days 0, 15, and 29. Muscle total creatine content increased by 20% after 6 days of supplementation at 20g/day, and remained elevated after ingestion of 2 g/day for a further 30 days. Without the maintenance dosage of 2g/day after the loading period, creatine levels gradually declined (at a rate of 0.43 mmol/kg dry wt./day) to pre-supplementation values after 30 d. A similar, but more gradual 20% increase in creatine was also noted in subjects who consumed 3 g/day of creatine. Muscle Pcr levels also increased significantly after 6 days in groups 1 and 2, and after 14 and 29 days in group 3. Thus, it was found that the ingestion of 3g/day over a period of 30 days was as effective at raising tissue levels as the ingestion of 20g/day for 6 days.

Casey et al.(1993) reported an increase in resting PCr in type I and II fibers after ingestion of 20g CrH20 for 5 days. Furthermore, changes in PCr in type II fibers before two bouts of 30 s maximal isokinetic cycling were positively correlated with changes in PCr degradation during exercise in this fiber type and changes in total work production. The results suggest that improvement in performance was a consequence of increased PCr availability in type II fibers.

Muscle creatine content has also been suggested to be related to concurrent carbohydrate consumption during the creatine supplementation period. Green et al. (1996) performed two

studies in order to investigate the creatine uptake with carbohydrate. Blood and urine samples were analyzed in 22 healthy male subjects. Those subjects who had consumed carbohydrate in solution along with creatine had significantly lower plasma creatine and urinary creatine than those subjects who had consumed only creatine, or no supplement. Muscle biopsy samples were obtained from 24 males before and after ingesting 5 g of creatine four times a day for 5 days (group1), and after ingesting 93 g of simple carbohydrate along with the same creatine dose (group 2). Supplementation resulted in a significant increase in Pcr and total creatine in both groups, however, group 2 had a significantly greater (60%) increase in total creatine compared to group 1. These data suggest that carbohydrate ingestion augments muscle creatine retention. Although no mechanism has been suggested, the researchers hypothesize that it appears to be insulin-mediated, as creatine supplementation with carbohydrate ingestion dramatically elevated serum insulin concentration in the study.

In summary, the literature suggests that dietary creatine enhances the muscle creatine level significantly. Although several doses have been examined across many time periods, age groups, and activity levels, 20g/day for 5-7 days has been consistently shown to maximize muscle creatine levels. Furthermore, 2g/day has also been shown to maintain the maximal level reached by an immediately previous loading period.

ACUTE CREATINE SUPPLEMENTATION AND EXERCISE PERFORMANCE

An abundance of studies have investigated the effect of creatine supplementation on exercise performance. This is not surprising considering the central role of creatine in skeletal muscle metabolism during exercise and considering that the availability of Pcr is believed to be a contributory factor to fatigue during high-intensity exercise (Balsom et al., 1994). Furthermore, if submaximal exercise is performed during the period of supplementation, muscle uptake of creatine was found to increase even more (Harris et al., 1992).

Following the work of Harris et al. (1992) who suggested that 20g/day for 5 d was sufficient to increase total muscle creatine content 126.8 to 148.6 mmol/kg dry wt, and Pcr from 84.2 $\pm$ 7.3 to 90.6 $\pm$ 4.8 mmol/kg dry wt, Greenhaff and colleages reported that total muscle peak torque production during bouts 2, 3, and 4 of 30 maximal voluntary knee extensions at a constant angular velocity of 180 degrees/s, interspersed with 60 s recovery periods, was enhanced following the same dose of creatine supplementation (20g/day) in 12 moderately active, male and female subjects. Not only was the muscle peak torque production within a bout improved throughout bouts 2-4, but the torque production during the final 10 contractions of bout 1 (838 Nm $\pm$ 85 to 885 Nm $\pm$ 95), and contractions 11-20 of the fifth and final exercise bout were also significantly increased after supplementation (544 Nm $\pm$ 59 to 581 Nm $\pm$ 67).

The finding that creatine supplementation could enhance performance of short duration, dynamic, high intensity, intermittent exercise was confirmed in the double-blind study by Balsom et al. (1993). The exercise protocol, 10 6-s bouts of high-intensity cycling at 130 rev/min and 140 rev/min, was performed by 16 healthy, untrained men before and after a 6 d administration period of 25 g/day of Cr.H2O or glucose. Each exercise bout was divided into 0-2 s, 2-4 s, and 4-6 s intervals. Those in the creatine group tended to maintain a higher mean pedalling frequency over the last few bouts of the 2-4 s interval. During the 4-6 s interval, the difference between the groups became significant, as did the difference between groups after the

7th bout. There were no significant differences in mean pedalling frequency between the 2 groups before the administration period.

Further studies have produced conflicting evidence concerning creatine supplementation and high-intensity exercise performance. After five days of supplementation at 20g/day, total work production insignificantly increased by 4% during 2 bouts of 30 s maximal isokinetic cycling in 9 untrained, male subjects (Casey et al. 1996). Febbraio et al. (1995) also found no significant increase in cycling endurance. Six untrained men performed four exercise trials, each consisting of four 1-min cycling bouts, interspersed by 1 min of rest, followed by a fifth bout to fatigue, all at a workload designed to require 115-125% of VO2 max. The testing occurred after a 5 d supplementation period of 20g/d and after a 28 d washout period. No difference was observed in exercise duration in the fifth work bout between supplemented and non-supplemented subjects.

In addition, 4 d of supplementation at 4 X 70 mg/kgBW/day had no effect on the peak power output, mean power output, end-power output, and percent power decline during seven cycle ergometer sprints in nine recreationally active males compared to 8 similar subjects who consumed a placebo (Barnett et al. 1996). Each 10 s sprint was separated by 30 s of passive recovery except for sprints five and six, which were separated by five minutes. In addition, five days of supplementation at 20g/day had no effect on the peak power, total work, and fatigue of 6 healthy, untrained males who performed 10 maximal sprints against a constant load of 111.8 N for 15 s (Cooke et al. 1995). These findings suggest that oral creatine supplementation does not positively affect several cycling performance parameters.

In competitive rowers, 5 days of creatine supplementation at 0.25g/kgBW resulted in an improvement in the1000-m rowing performance of 19 competitive male rowers (Rossiter et al. 1996). After supplementation with a placebo, a control group showed no differences in performance time (214.0 $\pm$ 30.9 to 214.1 $\pm$ 31.5 s), while 84.2% of the creatine subjects improved their performance times (211.0 $\pm$ 21.5 to 208.7 $\pm$ 21.8 s).

Studies have also been performed on swimmers. No differences in swimming sprint performance times of their best stroke in the 25-,50-and 100-m were observed after 5 days of supplementation at 20g/day in 10 highly-trained male and female swimmers (Mujika et al. 1996). Similar findings were reported by Burke et al. (1996). Thirty-two elite swimmers (18 males and 14 females, age 17-25) from the Australian Institute of Sport were tested on two occasions, 7 d apart. Tests performed were the 25-, 50- and 100-m maximal sprints, electronically timed with a dive start, and each interspersed with 10 min of active recovery. A 10 s maximal ergometry test was also performed. Subjects were administered 20g/d of creatine or placebo for 5 d. The results showed no significant differences between the group means for sprint times or maximal leg ergometry power and work. These studies are not supportive of creatine's ability to enhance single-effort performance.

Finally, running velocity has been suggested to be unaffected by creatine supplementation (Redondo et al. 1996). Eighteen highly-trained collegiate athletes sprinted three 60 m distance trials interspersed by 5 min rest periods. Subjects in the treatment group ingested creatine at 20g/day for 7 d, while the placebo group consumed glucose. Analysis of the velocities of the subjects through the 3 trials before and after supplementation indicated that there were no significant main or interaction effects between groups.

The hypothesis that supplementary creatine ingestion will improve high-intensity performance has not been consistently upheld by research. The literature reports several studies of acute supplementation having no effect on lower-body, high-intensity exercise as measured by treadmill running or cycle ergometry (Balsom et al.1993, Febbraio et al.1996, Odland et al.1994, Burke et al.1996, and Redondo et al.1995). The common aspect of these studies is the activity level of the subjects: all were highly trained or collegiate athletes engaged in regular training programs. It is possible that the fitness level of these subjects was at a near-maximal level such that creatine supplementation was ineffective. Harris et al. (1993) reports that some active subjects have muscle creatine stores close to the maximal level achieved by dietary supplementation as a result of training.

However, the improvements in performance observed during some high intensity exercise following creatine supplementation as demonstrated in several proceeding studies may be partly explained by a greater availability of PCr in the working muscle before each exercise period, perhaps due to a higher pre-exercise concentration, and/or a higher rate of resynthesis during recovery periods (Balsom et al., 1994).

CHRONIC CREATINE SUPPLEMENTATION AND EXERCISE PERFORMANCE

Few studies to date have investigated creatine supplementation for periods longer than 7 days. Earnest et al. (1995) examined the influence of chronic Cr.H2O supplementation for 28 days. Eight experienced weight-trained male subjects received either a glucose placebo or 20g/day of creatine for 6 days, and 2g/day for 22 days. Subjects were tested on 1RM bench press as a test of muscular strength, and lifting repetitions at 70% of 1RM bench press until fatigue. Lifting cadence was paced throught the the use of a metronome set at 1 s timing interval. Fatigue was defined as the inability to complete one lifting repetition, or the inability to maintain the lifting cadence. Bench press 1RM significantly increased 6% in the creatine group, while the placebo group remained unchanged throughout the 28 days. Lifting repetitions were also significantly increased (11.5 $\pm$ 0.8 to 15.5 $\pm$ 1.5 reps), compared to the control group which showed no change. Given these results, it is possible that chronic supplementation may cause greater strength increases than short-term supplementation. This experiment was the first to demonstrate that creatine supplementation improves certain strength training parameters, and suggests the efficacy of creatine as an ergogenic aid for resistance exercise.

Several unpublished studies have also found strength increases in chronically supplemented subjects. Ferreira et al.(1997) placed 25 collegiate football players into two groups, one which ingested Phosphagen HP (containing creatine, glucose, taurine, and electrolytes) according to manufacturer's recommendations, and the other which ingested a placebo of glucose, taurine, and electrolytes. Both groups supplemented their diets for 28 days while performing resistance training 5 h/week and 3 h/week of agility/sprint training. Subjects were tested before and after the administration period on a maximal repetition test at 70% 1RM bench press and upright squat in order to determine lifting volume. Results revealed that maximal lifting volume in the bench press was significantly enhanced, while squat lifting volume did not change. The mean change from baseline for lifting volume on the bench press in the creatine group was 222 $\pm$75 kg, while only -20.0 $\pm$ 37 kg for the placebo group. Similarly, Becque et al. (1997,abstract) found strength increases after 6 weeks of supplementation and training. Two groups (total n=23) of experienced weight lifters ingested either 20g/day for 7 d and 2g/d for 21 d,

or a placebo. Before and after 6 weeks of periodized strength training, both groups were tested on 1RM biceps curl strength. The 1RM for the treatment group increased from 42.8 + 17.7 kg to 54. 7 + 14.1 kg. While the placebo group increased also, the 1RM values increased significantly more for the creatine supplemented group.

Low dosages of creatine over a longer period of time have also been studied. Goldberg et al. (1997 abstract) examined the 1RM bench press and leg extension of 34 male varsity football and track athletes between 18-22 years. Subjects were given 3g/d of creatine or placebo for 14 d during an off-season weight training program and were tested before and after supplementation. Neither performance parameter was affected by the creatine supplementation. Thompson et al. (1996) also investigated the effects of chronic creatine supplementation by administering creatine at 2g/day for 6 weeks to collegiate swimmers in training. Magnetic resonance and spectroscopy were used to study the calf muscle metabolism in a group of female varsity swimmers at rest and during plantar flexion exercise. No differences were noted in muscle creatine concentration or performance between the creatine and placebo groups. However, these studies may not have affected performance because of the absence of a loading period which has been suggested to rapidly maximize creatine stores (Harris et al. 1994).

Muscle uptake of creatine has been shown to increase in subjects who have exercised during the creatine supplementation period. To study the interaction between exercise and creatine uptake, five subjects were given 20 g/day of creatine for 3.5 d. The subjects performed 1 h of bicycle ergometry using one leg, using the maximum resistance at which they could perform the task. The other leg was rested at this time and served as a control. Muscle biopsy samples were taken from the rested and un-rested vastus lateralis before and after the supplementation period. Exercise resulted in a mean increase in total creatine content in the control legs (118.1 $\pm$ 3.0 to 148.5 $\pm$ 5.2 mmol/kg dry wt) and a greater significant increase in the exercised legs (118.1 $\pm$ 3.0 to 162.2 $\pm$ 12.5 mmol/kg dry wt) after creatine supplementation. In four of the five subjects, the total creatine content exceeded 155 mmol/kg dry wt. The Pcr concentrations at the end of the suppplementation in the exercised legs averaged 103.1 $\pm$ 6.2 mmol/kg dry wt compared to 93.8 $\pm$ 4.0 mmol/kg dry wt in the control legs, and 81.9 $\pm$ 5.6 mmol/kg dry wt before supplementation.

Given the results of Harris et al. (1993), who found greater increases in total muscle creatine and performance in trained muscle, chronic supplementation with concurrent strength training may produce even greater improvements in strength performance. The ability to resynthesize Pcr in the muscle may be greater with higher total muscle creatine. Thus, recovery of Pcr between sets of exercises may be more rapid and allow higher work output in repeated bouts of exercise. After training, the increased levels of Pcr stores, may allow more total work to be done during resistance training sessions. This increased stimulus on the muscles may result in greater strength increases over time. However, few studies of this nature have been performed to date.

ADVERSE EFFECTS OF CREATINE SUPPLEMENTATION

Because creatine forms in vivo, by hydrolysis of creatine and Pcr, creatinine determinations in serum and urine are useful for evaluating kidney function. As an indicator of kidney function, creatinine clearance is often used as a factor to determine the dose of many drugs. Elimination rates of many hormones and toxic metabolites are compared with those of

creatinine. Harris et al. (1992) collected blood samples of their female and male subjects who ingested 20g/day for 4.5 d, 7 d, and 10 d, 30g/d for 7 d, and on alternate days for 21 d. These dosages represented a variety of situations in which to examine the blood profiles of creatine-supplemented subjects. They reported that hematology and routine biochemistry revealed that no changes occurred throughout the study for any subject at any of the dosages. However, no values were provide for these prameters.

Animal studies have suggested no ill effects of high creatine diets or long-term creatine feeding. Weanling rats were fed a high-creatine diet for 28 d and the kidney transamidinase levels after the feeding period suggested that no toxic effects of creatine were present (Walker et al. 1960).

To date, the only adverse effect of creatine supplementation has been an increase in body mass. However, most of these studies have involved large doses of creatine over short periods of time. Few long term creatine supplementation studies have been performed, thus it is not clear whether there are any adverse effects associated with its prolonged use. This suggests the importance of measuring some metabolic health indicators to assure the safety of chronic creatine supplementation.

CREATINE AND BODY COMPOSITION

It has been observed that creatine supplementation results in an increase in body weight. Earnest et al (1995) reported an increase of 1.7 kg in four experienced male weight training subjects who completed 28 of days of their habitual resistance exercises along with creatine monohydrate at the dose of 20g/day for 5 d and 3g/day for the remainder of the study. Balsom et al (1993) reported an increase in body mass of 1.1 kg in eight healthy male subjects after 6 d of creatine supplementation at 25 g/day for 6 d. Greenhaff et al. (1994) showed a body mass increase of 1.6 kg in 8 subjects after ingesting 20 g/day of creatine for 5 d. In addition to the previous study, Balsom et al. (1995) reported an increase of 0.9 kg in the body mass of seven male subjects after 6 d of creatine supplementation at 20 g/day. Although several researchers have demonstrated short term increases in body mass with acute creatine supplementation, there is a lack of information regarding change in body mass with chronic creatine supplementation concurrent with resistance training or the actual composition of weight gain.

Only one published study and several studies published only as abstracts have investigated the composition of weight gain. Earnest et al. reported an increase of 1.7 kg in total body weight, but no significant change in percent body fat or fat-free mass as assessed by hydrostatic weighing. Goldberg et al. (1997 abstract) found a significant difference between creatine and placebo supplementation groups after 14 d of ingestion and weight training of 34 male varsity football and track athletes. The creatine group increased 105.71 to 106.61 kg throughout the study. Becque et al. (1997 abstract) studied the body mass and fat-free mass of 23 experienced weight lifters under an ingestion regimen of 20 g/day for 7 d and 2 g/d for 35 d of creatine or a placebo. The subjects performed 6 weeks of periodized strength training. Body mass for the creatine group increased from 86.7 $\pm$ 14.7 kg to 88.7 $\pm$ 13.8 kg. A similar trend was noted in the fat-free mass of the creatine group, with the mean values increasing from 71.2 $\pm$ 10.0 kg to 72.8 $\pm$ 10.1 kg. Kreider et al (1997 abstract) revealed that the mean body mass increase of the creatine group was 2.22 $\pm$ 0.5 kg compared to 1.34 $\pm$ 0.8 kg in the control group in 25 varsity football players who ingested creatine or a placebo for 28 d throughout a period of activity (5

h/week of resistance training and 3 h/week of agility drills). Fat-free mass was also increased significantly in the creatine group (mean=2.43 ± 0.4 kg) compared to the control group (1.33 ± 0.3 kg). Stout et al (1997 abstract) also investigated weight gain in 24 varsity football players who ingested 20 g/day of creatine for 5 d, and 10 g/d for 52 d during a weight training regimen of lifting 4 h/week and 2 h/week of sprinting drills. The treatment group increased their fat-free mass by a mean of 2.6 ± 2.0 kg, while the control group increased by only 0.01 ± 2.6 kg.

Another study (Kreider et al. 1996) has reported body mass increases in 30 male weight-trained subjects who had ingested diet supplements containing 20 g creatine for 28 d. The control group ingested a non-fortified carbohydrate placebo. The subjects continued with their personal regular resistance training regimen throughout the study. Body mass increased significantly within 7 d compared to the control group and the pre-test values, and continued to increase significantly by day 14 and 28, both compared to the pre-test values and to the control group. Fat-free mass showed the same significant trend as body mass.

Balsom et al.(1993) proposed that the increases seen in body mass may be due to an increase in total body water content or an increased protein synthesis. Several theories support the role of creatine's involvement in muscle protein synthesis, however, the mechanism by which creatine acts is unknown. It is suggested that the primary effect is connected with the nucleus and is accomplished at the transcriptional level. Creatine may also act indirectly by increasing the hydration status of the cell which has been suggested to be a positive effector in regulating the biosynthesis of actin, myosin, and creatine kinase by developing muscle cells. Cell hydration is hypothesized to be an anabolic signal which may translate into increased fat-free mass over time in a healthy, resistance-trained individual (Kraemer et al.) The composition of weight gained must be identified in order to explain whether this is a benefit or detriment of creatine supplementation.

CREATINE AND BLOOD LACTATE

Creatine plays an integral role in energy metabolism as a substrate for the formation of Pcr. Pcr is the most rapid source of energy for ATP resynthesis from ADP through the creatine kinase reaction. A possible function of Pcr is to modulate glycolysis. During sudden intense muscular activity, the glycolytic flux may increase several hundredfold. Also, during this time, the concentration of Pcr is decreased due to the rapid need for replenishing ATP in the active muscle via the creatine kinase reaction. It is postulated that the key glycolytic enzyme, phosphofructokinase (PFK), is partially inhibited by physiological concentrations of Pcr (Greenhaff et al. 1994). During strenous activity, when the Pcr concentration is decreased, PFK is disinhibited and glycolysis is increased to provide ATP. Thus, a higher initial Pcr level may slow the activation of anaerobic glycolysis for resynthesis of ATP. Greenhaff et al (1994) suggested that an increased creatine level increases Pcr resynthesis which leads to an increased ability to maintain the free creatine concentration higher than the Km of creatine kinase for creatine, pushing the creatine kinase reaction toward Pcr resynthesis and ADP formation. A consequence of a decreased dependance on glycolysis is less accumulation of lactate and hydrogen ions.

An accumulation of hydrogen ions from lactate result in a decline of the muscle's force capabilities. (Volek et al.1996). The decline in power output observed during high intensity exercise may be the result of lactic acid accumulation in the muscle which dissociates to the

lactate anion and hydrogen cation, thereby reducing the pH of the muscle. Several mechanisms are involved with the effects of decreased pH on the muscle. The onset of fatigue may be associated with an increase in hydrogen which has a direct action on the contractile apparatus by affecting either calcium or myosin ATPase. Acidosis may also affect equilibrium reactions such as the creatine kinase reaction, resulting in a more rapid depletion of creatine phosphate. In addition, tension is reduced when the arterial blood and muscle lactate concentration is increased independent of a change in pH (Balsom et al. 1994). Elevated muscle Pcr may reduce the production of lactate as stated above and may also improve buffering of these hydrogen ions that are produced. Pcr acts to buffer protons which are products of ATP hydrolysis. When the creatine kinase reaction is in favor of ATP regeneration, protons are utilized. This buffering capacity helps prevent acidification of cells, allowing the muscle to generate a higher force for a longer period of time.

Several studies have documented the changes in blood lactate accumulation which occurred before and after creatine supplementation. Balsom et al. (1993) found that blood lactate accumulation (post-exercise value minus pre-exercise value) of 16 male subjects decreased significantly from 10.8 mmol/L before the 6 d administration period to 9.0 mmol/L after creatine supplementation in ten 6 s bouts of high-intensity cycling at 140 rev/min on a cycle ergometer with 30 s rest periods between each bout. In addition to decreased lactate, the creatine group was better able to maintain the pedal frequency during the second half of each bout, translating into a higher power output. Conversely, Greenhaff et al. found no difference when comparing blood lactate accumulation before and after 6 d of creatine supplementation in 12 physically active male subjects who performed five sets of 30 maximal voluntary knee extensions at a constant angular velocity of 180 deg/s on an isokinetic dynamometer. However, the creatine group showed greater torque production in bouts 2-4. Thus, higher work output (combined with no change or a decrease in blood lactate accumulation) suggests the efficacy of creatine in increasing pre-exercise Pcr levels. This, in turn, may facilitate an accelerated rate of Pcr resynthesis during the recovery periods between bouts.

Other research has observed less lactate accumulation in creatine compared to placebo groups along with improved power and/or work output. Eight male subjects performed five 6 s bouts at 140 rev/min on an cycle ergometer interspersed with 30 s rest periods, followed by one 10 s bout after a 40 s rest period, after 20g/d creatine for 6 d. As a result of the creatine supplementation, all subjects were better able to maintain target speed near the end of the 10 s bout. Mean blood lactate accumulation after the five bouts was significantly less in the creatine group (Soderlund et al. 1993). Using a protocol of three 30 s bouts of isokinetic cycling interspersed with 4-minute rest periods, Birch et al. (1995) examined peak and mean power output, total work output, and blood lactate accumulation following creatine supplementation. Although no differences were observed in blood lactate accumulation before and after creatine supplementation, peak power output was 8% higher during bout 1, and mean power output was 6% higher during bouts 1 and 2 after supplementation. Total work was also higher during bouts 1 and 2 for all supplemented subjects.

Several studies have noted no changes in either blood lactate or power and/or work output after creatine supplementation. Six untrained men performed four exercise trials, each consisting of four 1-min cycling bouts, interspersed by 1 min of rest, followed by a fifth bout to fatigue, all

at a workload designed to require 115-125% VO2 max. The testing occurred after a 5 d supplementation period of 20g/d and after a 28 d washout period. No significant differences were noted in lactate accumulation or cycling endurance after supplementation (Febbraio et al. 1995). Four days of supplementation at 4 X 70 mg/kgBW/day had no effect on the blood lactate accumulation, peak power output, mean power output, end-power output, and percent power decline during seven cycle ergometer sprints in nine recreationally active males compared to eight similar subjects who consumed a placebo. Each 10 s sprint was separated by 30 s of passive recovery except for sprints five and six, which were separated by five minutes. (Barnett et al. 1996). No differences in blood lactate or swimming sprint performance times of their best stroke in the 25-,50-,and 100-m were observed after 5 d of supplementation at 20g/day in 10 highly-trained male and female swimmers (Mujika et al. 1996).

In summary, creatine supplementation has not been conclusively shown to have a beneficial impact on blood lactate levels during and after exercise. The improvements in performance seen in several preceeding studies have been suggested to be a direct result of decreased blood lactate in creatine-supplemented subjects. Future research must determine the exact mechanisms by which these findings occur.

SUMMARY

Creatine was first identified in 1832 in meat extract, later it was extracted from several types of muscle but not other organs. The realization that creatine was more than a by-product of metabolism occurred when it was observed that a major portion of creatine was retained by the body. Since this time, much work has been devoted to the understanding of the biosynthesis of creatine and its pivotal role in the regulation of skeletal muscle energy metabolism and fatigue.

Skeletal muscle contraction and relaxation is fueled by the energy released upon dephosphorylation of ATP. Maximal intensity exercise of short duration relies almost exclusively on the creatine phosphate system whereby PCr is degraded anaerobically to release energy for rephosphorylation of ADP. It has been suggested that a decline in force production may be related to depleted PCr stores which may limit the rate of ADP rephosphorylation (Balsom et al., 1994). Thus, it is possible that increasing the total available PCr and Cr stores may reduce the rate of PCr depletion during maximal contraction and aid the rate of ADP rephosphorylation, translating into potentially increased maximal performance. Since this time, much work has been devoted to the understanding of the biosynthesis of creatine and its pivotal role in the regulation of skeletal muscle energy metabolism and fatigue.

Lately, much attention has been devoted to the use of creatine in order to enhance performance. Neither endurance exercise nor maximal oxygen uptake appears to be enhanced, nor have any adverse side effects of creatine supplementation been reported. A few studies have demonstrated increased maximal power output with creatine supplementation (Greenhaff et al., 1993, Balsom et al., 1993, Harris et al., 1993), while an equal number of studies have reported no impact of creatine supplementation on performance (Cooke et al., 1995, Terrillion et al., 1996, Ruden et al., 1996). A few studies have examined the effects of creatine supplementation on strength-trained subjects (Earnest et al., 1995, Ferreira et al. 1997, Thompson et al. 1996), and fewer studies have investigated if females may benefit from creatine supplementation. Discovering if chronic creatine supplementation can improve performance in strength-trained females may add to the debate regarding creatine as an ergogenic aid.

The literature regarding creatine as an ergogenic aid remains controversial, particularly with respect to chronic versus short-term supplementation, as well as its benefit for both the sexes. As creatine suplementation has been suggested to improve muscular strength, future research regarding creatine supplementation on maximal exercise may provide further evidence into the benefits of creatine supplementation.

CHAPTER III

JOURNAL MANUSCRIPT

TITLE: THE EFFECTS OF CHRONIC CREATINE SUPPLEMENTATION ON PERFORMANCE AND BODY COMPOSITION IN FEMALE ATHLETES

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ABSTRACT

The purpose of this investigation was to determine the effects of five weeks of creatine monohydrate ingestion on body composition, blood metabolite, and muscular performance measures in 16 female NCAA Division1 lacrosse players. Subjects were randomly divided into placebo (P,n=9) and creatine supplement (C,n=7) groups. The supplement group was administered 20g/d of creatine monohydrate in capsule form for 7 d and 2g/d thereafter for five weeks during which time the subjects were engaged in a pre-season conditioning program. Pre- and post-testing consisted of a three-site skinfold analysis, bioelectric impedence (BIA) measurements, hydrostatic weighing, isokinetic knee extension muscle endurance test (5 sets of 30 repetitions at 180 degrees/sec.), blood lactate response to the performance test (pre-test and 3 minutes post-test), a 1RM bench press and 1RM leg extension test. Pre-,mid-, and post- values of blood parameters (BUN and GPT) were measured in order to ensure the safety of the subjects. Data was analyzed using two-way ANOVA with repeated measures, and values are presented as mean±SEM for C and P groups, respectively. Testing revealed that 1RM bench press significantly increased in both groups (mean increase both groups: 4.5kg), and the C group improved significantly more than the P group (6.17±1.96 and 2.84± 1.84 kg). Percent body fat by skinfold also decreased significantly in both groups over time (0.52%), and the C group decreased their body fat significantly more than the P group (1.2+0.92 and +0.29+0.81%). Percent body water by BIA also decreased significantly in both groups over time (2.0%), and the C group decreased their percent body water significantly more than the P group (3.0± 1.06 and 1.0± 0.92 %). There was a trend for body fat measured by hydrostatic weighing to decrease for for both groups over the 5 weeks. Although no significant differences between groups were found in all other measures, significant time effects across groups were noted (values are absolute mean increase for both groups) for body weight (0.49±3.2kg), 1RM leg extension (1.36±4.1kg), BUN (0.07± 0.03mmol/L), total work across 5 bouts of isokinetic knee extension (283.5 ±387.3Watts), and fat-free mass by skinfold (0.70 ±1.18kg). These data indicate that a regimen of dietary creatine supplementation designed to increase total muscle Cr content significantly improved the 1RM bench press strength, and decreased the percent body fat as assessed by skinfold and the percent body water as assessed by BIA of a supplemented group more than a placebo group when all female subjects are engaged in a common resistance training program. Furthermore, chronic creatine supplementation appears to have no detrimental effect on blood metabolites which indicate kidney and liver function.

Key words: female athletes, strength training, creatine, body composition, lactate

INTRODUCTION

The majority of creatine found in the body is located in skeletal muscle. Recent published results demonstrate that oral creatine supplementation of 20g/day for more more than 5 d increases the total creatine content of the muscle up to 50% in untrained males, and is maintained at a maximal level with the ingestion of 2 g/d thereafter (Harris et al. 1993). Currently, this dietary supplement is being tested on a variety of different subjects to determine whether it can delay fatigue and/or improve performance parameters in which the creatine phosphate system is the primary source of energy (ie: high-intensity events). Some studies showing performance improvements have suggested that the ergogenic effect of creatine lies in the increased rate of ATP resynthesis during exercise, which is supported by decreased levels of blood lactate and/or plasma ammonia accumulation. Balsom et al. (1993)found that blood lactate accumulation (post-exercise value minus pre-exercise value) decreased significantly after creatine supplementation, and the creatine group was better able to maintain the pedal frequency during the second half of each isokinetic knee extension bout, translating into a higher power output. Soderlund et al.(1993) found that all subjects were better able to maintain target speed near the end of a cycle ergometry bout. In addition, mean blood lactate accumulation after the five bouts was significantly less in the creatine group.

Other reports in the literature show no benefit of creatine supplementation and/or no changes in blood metabolites. Supplementation had no effect on the blood lactate accumulation, peak power output, mean power output, end-power output, and percent power decline during cycle ergometer sprints (Barnett et al. 1996). No differences in blood lactate or swimming sprint performance times was observed in 10 highly-trained male and female swimmers after supplementation (Mujika et al. 1996).

In addition to improved performance, several studies have noted changes in body composition related to creatine supplementation with increases from 1.1 to 1.7 kg over a period of 1-4 weeks (Earnest et al.1995, Balsom et al 1993, and Greenhaff et al. 1994). The composition of the weight gain is hypothesized to be related to an increase in total body water, however, very few studies have examined this. Kreider et al.(1997 abstract) found significant increases in body weight and fat-free mass in collegiate football players. The increases were not validated by increases in total body water, nor is it clear whether creatine alone was responsible for the results. Terrillion et al.(1996) found that creatine did not alter body weight or body water in male runners who supplemented for 5 d. There have been no published studies examining body composition changes with chronic supplementation using female subjects.

Most studies testing the effects of creatine supplementation have used cycle ergometry (Balsom et al1993), treadmill running (Balsom et al 1993), isokinetic dynamometers (Greenhaff et al 1993), and/or muscle endurance tests (5 sets of 10 repetitions at 70% 1RM)(Earnest et al.1995). Few published studies (and several studies published as abstracts) have examined the effects of chronic creatine supplementation during resistance training. In addition, the majority of studies examining creatine supplementation have used only male subjects. A few studies have combined male and female subjects (Redondo et al.1995, Greenhaff et al.1993) but did not

analyze effects seperately by sex. Thus, additional research is required to examine the effects of long-term creatine supplementation in highly-trained female athletes.

In summary, previous research has suggested that creatine supplementation may improve anaerobic exercise performance in males. It is not clear whether there is also a benefit of this supplement for females. Most studies have been done using short-term supplementation (in spite of the fact that most athletes use it over a long-term period), but there is some evidence that there may be a positive effect of long-term supplementation with concurrent strength training. This study examined the effect of chronic creatine supplementation on performance and body composition of female athletes involved in regular resistance training, while also measuring some blood factors to ensure safety of longer term creatine supplementation.

METHODOLOGY

SUBJECT SELECTION AND SCREENING

The trained subjects were 20 NCAA Div.1 female lacrosse players between the ages of 18-22 who had been medically cleared by a team physician. At the time of this study (October-November 1996), the subjects were in off-season training. Subjects were excluded if they had taken creatine supplements within the last two months. This subject pool was chosen since they are required to be involved in a common resistance training program over 5 weeks, and they were likely to be highly motivated to improve performance and comply with study guidelines. Subjects were randomly distributed between two groups by the secondary investigator. The study was double blind. Permission was obtained for this study by the Internal Review Board for research involving human subjects at Virginia Tech.

Following completion of an informed consent, each subject completed a survey requesting information about dietary habits (particularly vegetarianism and dieting for weight loss), body weight history, and general health. Throughout the supplementation period, the subjects continued their regular exercise training protocol which consisted of a free weight bench press and Nautilus leg extension cycle based on each subject's 1RM values. Week 1 consisted of 4 sets of 10 repetitions at 40-60% of 1RM. There was a progression over the five weeks of intensity and number of sets such that 5 sets were performed at 50, 75, 80, and 85% of 1RM (10 reps for the first set and 5 reps for the other sets). In addition, the subjects complemented their resistance training with other free weight exercises such as bicep curls (standing, barbell raised to shoulder), wrist curls (hand goes through 180 degree motion with barbell), jump shrugs (standing shoulder shrugs with bar), and medicine ball throws (ball thrown from chest outward) using the maximum loads in which they were able to perform the prescribed sets and repetitions (2-6 sets of 5-10 repetitions). The exercise prescription and training was the same for all subjects, which required them to train on Monday, Wednesday, and Friday of each week.

Compliance for inclusion in this study was based on accurate ingestion of the prescribed creatine or placebo, attendance and adherence for all testing procedures, and a minimum of 85% attendance at the required strength training sessions (a sign-in sheet was kept in order to monitor attendance).

Two subjects were dismissed from the study because of illness which caused them to miss more than the allotted weight training absences. Another subject became injured and her strength training program was modified. The final subject who dropped out of the study did so

for personal reasons. Therefore, the final number of subjects in each group was 7 in the creatine group, and 9 in the control group.

MEASUREMENTS

The design of the study with the schedule of measurements is given in Table 1.

BODY COMPOSITION: Body weight was assessed to the nearest 0.1 kg on a scale on day 1 and 2, and again on day 45 at the same time of day after an overnight fast on all three days. Percent body fat was determined by hydrodensitometry (Novel Products) with a load cell on day 1 and 45. The three highest under water weights from eight measurements were averaged to calculate body density which was was then inserted into Siri's equation to calculate percent body fat. Residual volume of the lungs was measured using the procedure as described by Wilmore (1970). Percent body fat by skinfold was assessed on day 1 and 45 using the equation by Jackson et al. (1980) to calculate body density which is then used in Siri's equation to calculate percent body fat. The RJL 101A-BIA bioelectric impedence machine was used to analyze body water on day 1 and day 44 after an overnight fast. Body water and percent body fat by BIA was calculated using the software provided by RJL.

MUSCULAR STRENGTH: A 1RM free weight bench press (Nautilus) and 1RM leg extension (Nautilus) test was used to determine muscular strength on day 3 and day 46. The subjects warmed-up by performing 2 sets of 5 reps at 50% of their maximum weight, followed by stretcing. The subjects began the test at 85% of their previous 1RM, however, an average of 3 trials was needed to reach a 1RM for all subjects. They were allowed 3 minutes of to rest between trials. Criteria for a full repetition on the leg extension included proper technique and a body angle such that the upper leg (thigh) was 90 degrees from the floor. Criteria for a full repetition on the bench press included proper technique (hips are never lifted off the flat bench), and full arm extension.

WORK FATIGUE and TOTAL WORK: A test of 5 X 30 maximal voluntary unilateral knee extensions (Biodex isokinetic dynamometer, Biodex Corporation, Shirley, NY) at a constant angular velocity of 180/sec, separated by a 1 min. recovery period were performed on day 1 (familiarization and orientation only) and day 2 (prior to ingestion of the first creatine supplement) and day 45 at the same time of day, 24 hours after the muscle strength tests, and after an overnight fast. The warm-up included walking in place and stretching for several minutes. The subjects sat in the Biodex chair and their dominant leg was attached securely to the measurement arm of the Biodex machine. While they performed the repetitions, they received verbal encouragement to perform as best as they could. The aim of this protocol was to induce fatigue across the five bouts. Work fatigue is defined as the work output in the last third of a bout subtracted from the work output in first third of the same bout, divided by the work output in the first third of a bout, and was assessed for each of the five sets. Work fatigue for this study was defined as the work fatigue in the last bout as a percent of work fatigue in the first bout. Total work is defined as the total work output measured per set across the five bouts.

BLOOD METABOLITES: Blood lactate (YSI Model 1500 Sidekick analyzer, Yellow Springs, OH), was obtained on day 2 and day 45 from fingerprick blood samples at rest and 3 minutes after the fifth exercise set. Blood was collected into capillary tubes in duplicate and run in duplicate. Blood lactate accumulation was calculated by subtracting the initial from the final concentration. Resting blood GPT (colorimetric, Sigma diagnostics #545) and blood urea nitrogen (colorimetric, Sigma diagnostics #66) were taken from venous blood samples collected on day 3, day 19, and day 46 (after an overnight fast) to assess liver and kidney function. Both assays

were analyzed in triplicate. A certified medical laboratory technician from the Department of Human Nutrition and Foods took a blood sample from each subject's arm. The samples were centrifuged to obtain serum, labeled and frozen at -20 degrees C until analysis.

CREATINE OR PLACEBO ADMINISTRATION

The study was double-blind in that one group received 4*5g/day of creatine monohydrate in capsules (form of dietary creatine produced and donated for this study by SportPharma Inc.) for 7 days (day 4 to day 10), with 2g/day for the remainder of the study (day 11 to day 46), while the other group received a placebo (sucrose) of similar appearance for the same time period. The subjects were instructed to ingest the creatine with plenty of water, and at least 3 hours apart during the loading phase. During the maintenance phase, the subjects were instructed to ingest the capsules at the same time of day for the remainder of the study. The subjects were given the amount required for the 7 d loading period prior to the ingestion period, and they were given the remainder of the capsules after one week of loading. A blank calendar was given to all subjects as a daily reminder and they were instructed to note the time of day the creatine was taken. In addition, the principle investigator was in contact with the subjects everyday throughout the study and verbally reminded the subjects of their responsibilities with respect to creatine ingestion and/or testing. Prior to all testing except the muscular strength tests, the subjects were instructed to fast for 12 h, and to avoid caffeine and alcohol for 24 h before testing. For the 1RM bench press and 1 RM leg extension tests, subjects were instructed to eat at least 3 h prior to the test, and also avoid caffeine and alcohol for 24 h.

DATA ANALYSIS

A two-way ANOVA with repeated measures was used to determine differences between the placebo and treatment groups for the following dependent measures: 1RM bench press, 1RM leg extension, work fatigue, total work, blood lactate accumulation, body weight, fat-free mass by skinfold and hydrostatic techniques, body water by BIA, and percent body fat by skinfold, BIA, and hydrostatic weighing techniques. A two-way ANOVA (2X3) was used for blood metabolites GPT and BUN which were collected pre-, mid-, and post-test. Initial differences between treatment and control groups for all dependent measures were assessed concurrent with the two-way ANOVA determinations. Correlation between pre- and post-percent body fat by skinfold and pre-and post- percent body fat by hydrostatic weighing was done to assess validity of procedures.

Statistical analysis was performed on SPSS. Correlation between pre-percent body fat by skinfold and pre-percent body fat by hydrostatic weighing was done using Minitab Statistical Analysis. Throughout the data analyses, the significance was set at an alpha level of 0.05 in order to reject the null hypotheses.

RESULTS

The descriptive characteristics of the subjects is provided in Table 2. At the time of the study all subjects were enrolled at a Division 1 university on partial or full lacrosse scholarships. There were no significant differences in initial age, height, or weight of subjects between the two groups. Self-described information from a diet-health questionnaire revealed that 59% of subjects considered themselves vegetarian, while an additional 24% consumed red meat and/or

fish less than twice per month. At the time of the study, no subjects were attempting to lose weight, and the average weight fluctuation over the past 12 months was 0.35 (±SEM 0.89) kg.

Muscular Strength

There were no initial differences between groups for 1RM bench press or leg extension. There was a significant mean increase of 2.9 kg (7.63% of initial) in strength for bench press strength for both groups over time. 87.5% of subjects increased their bench press 1RM (range of increase: 2.3-9.1 kg). There was a significant interaction of group and time with a mean increase in the creatine group of 6.17 kg (16.67% increase over initial), while the control group increased by a mean of 2.84 kg (7.09% increase over initial). (Figure 1).

There was no significant effect of group nor a group by time interaction for leg extension 1RM (Figure 2). There was, however, a significant change in leg strength for both groups combined across time. The combined groups increased their leg extension by a mean of 1.36 kg (3.26% of initial).

Total Work and Work Fatigue

No initial differences were noted between groups for total work or work fatigue during the muscular performance test. There was no significant main effect of group or time, nor a significant interaction of group and time for work fatigue (Table 3). There was a significant time effect across groups for total work: there was an mean increase of 3.55% across 5 bouts in both groups: 9 subjects increased their total work production by an average of 283.61 Watts. No significant interaction between time and group was seen in total work across 5 bouts (Figure 3).

Body Composition

There were no initial differences between groups for body weight, fat-free mass or percent body fat by skinfold or hydrostatic weighing techniques. Although there was a significant increase in body weight for both groups (0.49kg), no group effect nor interaction between time and group was seen for body weight (Figure 4). There was a significant time effect and a significant interaction of groups over time for percent body fat by skinfold technique: 85.7% of subjects in the creatine group decreased their body fat, in contrast only 33.3% of subjects in the control group reduced their body fat (Table 4). The mean decrease for percent body fat by skinfold was 1.2% for the treatment group, while the control group increased their average body fat by a mean of 0.3. Although there was no significant interaction or group effects for fat-free mass by skinfold noted, the data from both groups across time reveals a significant increase in fat-free mass. In addition, 85.7% of creatine-supplemented subjects increased their fat-free mass by skinfold (range 0.35-3.27 kg), while only 44.4% of control subjects had a gain of fat-free mass over the experiment (range 0.91-1.15 kg) (Table 4).

There was no group effect or significant interaction between group and time for percent body fat or fat-free mass by hydrostatic weighing, however, there was a trend for time effect noted for a decrease in percent body fat by hydrostatic weighing ($p=0.112$) (Table 4). A correlation analysis revealed a mean correlation of 0.80 for all subjects between pre-skinfold percent body fat and pre-hydrostatic weighing percent bodyfat, and a mean correlation of 0.77 for post-skinfold percent body fat and post-hydrostatic weighing percent body fat. Percent body fat by BIA was also calculated, although the values obtained were not valid (Table 4).

No initial differences between groups was seen for percent body water by BIA. Although there was no group effect, there was a significant decrease in percent body water for

both groups combined. The mean decrease for both groups was 2.0%. There was a significant interaction between group and time, as the creatine group decreased their percent body water by 3%, while the control group decreased by 1% (Table 4). However, the validity of this measurement is suspect based on the unreasonable body fat values obtained using BIA.

Blood Metabolites

There were no initial differences between groups for blood BUN or GPT. No group, time, nor interaction between these factors was seen for GPT (Table 5). Although there were no significant differences across groups nor interaction over time for BUN (Figure 5), there was a trend ($p=0.062$) for increased BUN at the end of the study compared to pre- or mid-training values (Table 5). The mean BUN of the creatine group increased 0.05mmol/L from the original value, while the control group BUN mean increased 0.09mmol/L from the original value. All subjects at each of the three blood draw times had BUN values slightly below the expected range of 1.78-6.07 mmol/L.

There were no initial differences between groups for blood lactate accumulation. Although there were no significant differences in lactate accumulation over time after the muscular performance test, there was a trend for decreased blood lactate accumulation in the control group throughout the experimental period ($p=0.067$) (Figure 5) (Table 6).

Additional Measures

Comparisions were made between the strength increases of self-described vegetarians and non-vegetarians. The mean increase in 1RM bench press for vegetarians (n=3) in the creatine group was 6.04 ± 1.67 kg. The mean increase for non-vegetarians was 5.11 ± 2.39 kg, which was insignificantly different from the improvements in the creatine group. A correlation between increase in fat-free mass and increase in 1RM bench press of the creatine and placebo groups showed no correlation between the two measures for either group.

DISCUSSION

The major findings of this experiment are that a group of female athletes increased their bench press strength and decreased their percent body fat as measured by skinfold technique and percent body water as assessed by BIA, more over 5 weeks after a regimen of oral creatine supplementation than those doing the same resistance exercise program and taking a placebo. Although an increase in muscle creatine was not validated, the dose used has been previously shown to increase muscle Pcr and Cr content in men (Harris et al. 1993).

Muscular Strength

The significant increase in strength in both groups over time suggests the efficacy of the strength training protocol. The upper body strength increase in the treatment group of 16.67% ($p<0.05$) compared to 7.09% ($p>0.05$) (Table 1) in the control group found in this study is consistent with several studies which report similar gains. Earnest et al.(1995) found that 1RM bench press strength increased by 6% ($p=<0.06$) over a 28 d creatine loading and maintenance period in male weight lifters compared to the placebo group, whose 1RM decreased by 3%. In addition, that research group reported that total bench press lifting volume as measured by the number of lifting repetitions at 70% of 1RM increased by 31% ($p=<0.05$) in the creatine group, compared to a 4% decreased in the placebo group. Becque et al.(1997 abstract) observed an increase in 1RM bicep curl in experienced male weight lifters after 6 weeks of strength training,

also supportive of the ability of chronic creatine supplementation to enhance upper body strength. Similar to the present study, Becque found that both groups increased their 1RM strength, however, the creatine group increased more than the control group ($p<0.05$). The 1 RM for the creatine group increased by 22% ($p<0.05$), while the 1RM for the control group increased by 14% ($p<0.05$). No lower body performance parameters were measured in either of these studies. Similar to the present study, both the previous investigations involved highly-trained individuals who ingested a similar dose of creatine (20-30 g/d) for 4-6 weeks and were evaluated on 1RM, upper body strength measures. However, both of the other studies used male subjects while our data was from female subjects.

Other studies involving the effect of long-term creatine supplementation on both upper and lower body performance parameters are conflicting, and involve the addition of glucose in the supplements. Ferreira et al.(1997 abstract) found increases in upper body endurance in collegiate football players as measured by a maximal repetition bench press at 70% 1RM. The subjects participated in their prescribed resistance training program while ingesting a commercial supplement containing creatine and glucose or placebo for 28 d. While upper body strength was significantly enhanced in the study, back squat lifting volume did not change. Maximal lifting volume in the bench press in the creatine group increased by 222 $\pm$ 75 kg, while the control group decreased by 20 $\pm$ 37 kg. Similar to the present study, highly trained athletes were examined before and after their prescribed 4-week strength training regimen, and strength increases were found only in the upper body measures. However, differences in design from our study are that the supplement included 99 g/d of glucose, the creatine dosage within the supplement was 15.75g/d, and the subjects were male. In another study, Stout et al.(1997 abstract) examined the 1RM bench press, vertical jump, and 100-yard dash in collegiate football players before and after 8 weeks of strength training and supplementation with creatine and glucose, creatine only, or a placebo. All measures increased significantly in the creatine and glucose group, demonstrating that upper and lower body performance was enhanced by ingesting glucose with creatine. Creatine supplementation alone did not show any benefits for performance. Since we did not include a group consuming glucose with creatine, we cannot determine the effects of this combination supplement in women.

Short term creatine supplementation has also been suggested to improve upper and/or lower body endurance. Balsom et al.(1993), Soderlund et al. (1995), and Greenhaff et al.(1994) have all shown that high-intensity exercise performance on a cycle ergometer was improved after ingesting 20-25g/d for 5-6 days. Kraemer et al. (1995) found that following one week of either 25 g/d of creatine supplementation or placebo, the creatine group significantly outperformed the placebo group in a bench press performance test of 5 sets at 10RM. After one week, the creatine group performed 7.4 more bench presses. Peak power output during jump squats for 5 sets of 10 repetitions at 30% 1RM for the creatine group also increased significantly.

Other studies have shown no benefit of creatine supplementation on strength training. Goldberg et al.(1997 abstract) placed collegiate football and track athletes on a low dose regimen of creatine (3 g/d) for 14 d during their off-season training program. Results showed that performance in a 1RM bench press, forty-yard sprint, leg extension and leg sled were not enhanced, perhaps due to the lower dose of creatine for a shorter period of time than other studies which have found strength increases. A possible reason for differences in the effect of

creatine between these studies may relate to the training level of the subjects. Most studies which used less trained subjects found strength benefits.

The literature reports several studies of acute supplementation having no significant effect on lower-body, high-intensity exercise as measured by treadmill running or cycle ergometry (Balsom et al.1993, Febbraio et al.1996, Odland et al.1994, and Redondo et al.1995). The common aspect of these studies is the activity level of the subjects: all were highly trained or collegiate athletes engaged in regular training programs, also similar to our study. Creatine levels have been found to be elevated in exercised quadriceps muscles (Harris et al.1993), thus, supplementation may not have an immediate effect on Pcr levels and resynthesis. Over time, however, training at higher levels of intensity as a result of the increased Pcr availability may result in strength gains.

The reason for an increase in upper body strength seen in the present study is difficult to explain as lower body strength did not increase as a direct result of creatine. This may be due to the fact that females are relatively weaker in their upper bodies in comparison to their lower bodies. Thus, there may have been more potential for increased creatine levels in the upper body muscles, as those muscles are less developed in female lacrosse players. It has also been shown by one group of researchers that females have higher levels of quadricep muscle creatine per tissue than males (Forsberg et al. 1991), thus creatine supplementation may have been unable to significantly augment the intially high levels of creatine in the quadriceps muscles. It is also possible that the previous physical conditioning of the subjects brought them near their maximum potential in lower body strength and therefore were unaffected by creatine supplementation.

Several explanations may be offered for the increased strength seen in the creatine group of this study and others whose results are similar. Throughout the 5 week training period, the Pcr and Cr stores may have been elevated significantly, as suggested by Harris et al. (1993). The ability to resynthesize Pcr in the muscle may be greater with higher total muscle creatine. During each strength training session, the recovery of Pcr between sets of exercises may have been facilitated. This may have allowed higher work output in repeated bouts of exercise by achieving greater anaerobic ATP production, as suggested by Greenhaff et al. (1994). Thus, the increased Pcr stores may have allowed more total work to be done each session. The increased stimulus on the muscles may have resulted in greater strength increases over time.

There is also evidence that Cr is a positive effector in regulating muscle protein synthesis, however, the mechanism by which Cr acts is unknown (Volek et al. 1996). Balsom et al. (1993) found increased diameter of type II fibers found in patients supplemented with low dosages of Cr for 1 year. They postulated that this may have been due to increased synthesis of contractile protein (Balsom et al.1993). Ingwall et al. (1974) suggest that Cr may trigger increased muscular activity and protein synthesis simultaneously. Furthermore, they suggest that Cr may act as a transcriptional or translational factor, or may alter levels of amino acids necessary for muscle protein synthesis. Bessman et al. (1990) suggest that more Pcr is available for protein synthesis because of increased contractile activity and increased Pcr transport.

Total Work and Work Fatigue

The subjects could do 3.5% more work, an average of 283.6 Watts, in the five sets of 30 repetitions on the Biodex in the final performance test compared to baseline. However, there was no differencce in this improvement due to creatine supplementation. This exercise protocol

for testing muscular performance, identical to that previously described by Greenhaff et al. (1993), was chosen as one bout of 30 repetitions has been shown to produce mixed-fiber muscle metabolite responses similar to those observed during short-term maximal voluntary exercise (Cheetham et al.1986). Contraction at a rate of 180 degrees/s has also been shown to result in the recruitment of type I and type II fibers (Tesch et al. 1989).

The lack of effect of creatine on this muscle performance test of the present study are in conflict with those of Greenhaff et al.(1993) who used the same isokinetic protocol. Greenhaff and colleages reported that total muscle peak torque production during bouts 2, 3, and 4 of 30 maximal voluntary knee extensions at a constant angular velocity of 180 degrees/s, interspersed with 60 s recovery periods, was enhanced following creatine supplementation (20g/day) for 5 d in 12 moderately active, male and female subjects. Not only was the muscle peak torque production within a bout improved throughout bouts 2-4, but the absolute torque production during the final 10 contractions of bout 1, and contractions 11-20 of the fifth and final exercise bout were also significantly increased after supplementation. Thus, the findings reported by Greenhaff et al. are in conflict with those of the present study, perhaps as a result of the training history of the subjects. Those in the Greenhaff study were not highly trained, and thus may have significantly improved as a result of initial lower levels of Pcr and Cr in the muscle being dramatically elevated by supplementation. Harris et al. (1993) has reported lower levels of creatine in less active quadriceps muscle, and near maximal levels in highly trained subjects. The quadriceps muscles of the subjects in the present study may not have been affected by supplementation as their training level may have previously augmented muscle creatine stores.

High-intensity, lower body exercise has also been examined by several researchers. Balsom et al. (1993) investigated cycle ergometry parameters. The exercise protocol, 10 6-s bouts of high-intensity cycling at 130 rev/min and 140 rev/min, was performed by 16 healthy men before and after a 6 d administration period of 25 g/day of Cr.H2O or glucose. Each exercise bout was divided into 0-2 s, 2-4 s, and 4-6 s intervals. Those in the creatine group tended to maintain a higher mean pedalling frequency over the last few bouts of the 2-4 s interval. During the 4-6 s interval, the difference between the groups became significant, as did the difference between groups after the 7th bout. As in the Greenhaff study, the subjects were not highly trained, and the results are again in conflict with those of the present study. Similar to the present study, however, studies which have used highly trained subjects consistently show no improvements in lower-body, high-intensity exercise performance (Balsom et al.1995, Febbraio et al.1996, Odland et al.1994, and Redondo et al.1995). However, these studies only investigated the effects of 5-7 d of supplementation. No studies have examined how long term supplementation affects high intensity, lower body, isokinetic exercise.

Body Composition

A significant gain in body weight across groups occurred due to the strength training and was not influenced by creatine supplementation. Only one published study and several studies published only as abstracts have investigated body composition changes with chronic creatine supplementation. Earnest et al. reported a significant increase of 2% in total body weight for the creatine group, and no change in the control group after 28d of creatine supplementation and resistance training. Goldberg et al. (1997 abstract) found a significant difference in body weight between creatine and placebo supplementation groups after 14 d of 3g/d ingestion and weight

training of 34 male varsity football and track athletes. The creatine group significantly increased from a mean of 105.71 to 106.61 kg throughout the study, suggesting that even low dose supplementation may be effective in increasing body weight in trained athletes (although no published studies have been performed to support this). Becque et al. (1997 abstract) studied the body mass of 23 experienced weight lifters under an ingestion regimen of 20 g/day for 7 d and 2 g/d for 35 d of creatine or a placebo. The subjects performed 6 weeks of periodized strength training, and found that body mass for the creatine group increased from 86.7 $\pm$ 14.7 kg to 88.7 $\pm$ 13.8 kg.

Only one published study and several studies published only as abstracts have investigated body composition changes with chronic creatine supplementation. Many authors hypothesize that the increase seen in body weight is due to an increase in body water, however, very few studies have examined this. Kreider et al.(1997 abstract) found significant increases in body weight and fat-free mass in collegiate football players after 28 d of supplementation with creatine and glucose. The increases were not validated by increases in total body water, nor is it clear whether creatine alone was responsible for the results. Terrillion et al.(1996) found that creatine did not alter body weight or body water in five male runners who supplemented for 5 d. Our study suggests that body water was not responsible for the insignificant body weight increases in our subjects.

Percent bodyfat decreased and fat-free mass increased as measured by skinfold technique in both groups, most likely due to the strength training program. In addition, the creatine group significantly reduced their body fat more than the control group as a result of the creatine. This may have been due to the increased reliance on body fat of the supplemented subjects, however, as diet was not recorded, this cannot be definitive.

Earnest et al. (1995) reported no significant changes in percent body fat by hydrostatic weighing nor fat-free mass by hydrostatic weighing after 28d of creatine supplementation and resistance training. Becque et al. (1997 abstract) studied the fat-free mass of 23 experienced weight lifters under an ingestion regimen of 20 g/day for 7 d and 2 g/d for 35 d of creatine or a placebo. The subjects performed 6 weeks of periodized strength training, and found that the fat-free mass of the creatine group significantly increased from 71.2 $\pm$ 10.0 kg to 72.8 $\pm$ 10.1 kg. Similar to the present study, no changes were found in percent bodyfat by hydrostatic weighing. Kreider et al (1997 abstract) revealed that fat-free mass increased significantly in the creatine group (mean 2.43 $\pm$ 0.4 kg) compared to the control group (1.33 $\pm$ 0.3 kg) in 25 varsity football players who ingested creatine or a placebo for 28 d throughout a period of activity (5 h/week of resistance training and 3 h/week of agility drills). Stout et al (1997 abstract) investigated body composition in 24 varsity football players who ingested 20 g/day of creatine for 5 d, and 10 g/d for 52 d during a weight training regimen of lifting 4 h/week and 2 h/week of sprinting drills. The treatment group significantly increased their fat-free mass by a mean of 2.6 $\pm$ 2.0 kg, while the control group increased by only 0.01 $\pm$ 2.6 kg.

Additional analysis of percent body fat by BIA suggested that the BIA system is not a reliable measure. The percent body fat in the creatine and control groups increased substantially, and the means were out of realistic ranges for the subjects in the experiment.

Greater pre-exercise Pcr levels and resynthesis between exercise bouts may have led to increased protein synthesis, greater muscle mass, and thus metabolic rate. Although diet

analysis was not done, if diet remained the same, an increase in metabolic rate would increase reliance on bodyfat for energy.

Blood Metabolites

Blood metabolites were monitored throughout the experimental period in order to determine the effects of large doses of creatine on kidney and liver function. Although no side effects of creatine are reported in the literature, only one study (Harris et al.1993) reported taking similar precautions and found no changes in blood profiles. The investigation of GPT is undertaken in clinical enzymology as an index to various types of obstructive and infiltrative hepatic disorders. The BUN assay detects increased protein breakdown and renal disease with elevated values, and liver failure and increased protein anabolism with decreased values.

As previously reported in the literature, no changes were seen for blood GPT values throughout this study (Figure) and all subjects remained within the range of expected values. The trend ($p=0.062$) seen for increased BUN at the end of the study (Figure) may be a result of dehydration, which is supported by our body water data. The BUN values slightly below the expected range of 1.78-6.07 mmol/L could be due to increased plasma volume caused by adaptation to training.

The lack of effect of time or interaction of groups over time for blood lactate accumulation ($p=0.067$) (Table) suggests that no benefit was seen from the strength training or the creatine. Several other studies have found that lactate levels are not altered with creatine supplementation. Febbraio et al(1996) had six untrained men performed four exercise trials, each consisting of four 1-min cycling bouts, interspersed by 1 min of rest, followed by a fifth bout to fatigue, all at a workload designed to require 115-125% VO2 max. The testing occurred after a 5 d supplementation period of 20g/d and after a 28 d washout period. No significant differences were noted in lactate accumulation or cycling endurance after supplementation (Febbraio et al. 1995). Four days of supplementation at 4 X 70 mg/kgBW/day had no effect on the blood lactate accumulation, peak power output, mean power output, end-power output, and percent power decline during seven cycle ergometer sprints in nine recreationally active males compared to eight similar subjects who consumed a placebo. Each 10 s sprint was separated by 30 s of passive recovery except for sprints five and six, which were separated by five minutes. (Barnett et al. 1996). No differences in blood lactate or swimming sprint performance times of their best stroke in the 25-,50-,and 100-m were observed after 5 d of supplementation at 20g/day in 10 highly-trained male and female swimmers (Mujika et al. 1996).

As no studies have investigated blood lactate levels in chronically supplemented, highly trained subjects, only one direct comparison can be made to reports in the literature. Greenhaff et al.(1993) used the same isokinetic knee extension test performed in this study and compared blood lactate levels after warm-up, at the completion of each set, and 0, 2, 5, 7, and 10 min. after exercise in subjects who supplemented with creatine for 6 d. Muscle peak torque production improved during the final 10 contractions of each set, but no changes were found in blood lactate at any time throughout the performance test. Birch et al. (1995) also reported performance gains without changes in lactate levels. Thus, higher work output (combined with no change or a decrease in blood lactate accumulation) suggests the efficacy of creatine in increasing reliance on Pcr for ATP regeneration. Further research needs to determine the role of creatine supplementation on anaerobic glycolysis during exercise.

Additional Measures

Comparisions were made between the strength increases of self-described vegetarians and non-vegetarians. The 1RM bench press strength increases were not different between vegetarians and non-vegetarians, suggesting that initial levels of creatine in the muscles was not related to their diet, nor were the strength increases. However, the low number of subjects makes any conclusion about vegetarianism as a factor in strength improvement tenous. A correlation between increase in fat-free mass and increase in 1RM bench press of the creatine and placebo groups showed no correlation between the two measures for either group. Thus, strength increases were not always accompanied by decreases in fat-free mass.

In summary, the present experiment demonstrates that a regimen of dietary creatine supplementation designed to increase total muscle Cr content, significantly improved 1RM bench press strength and decreased percent bodyfat by skinfold more in female athletes engaged in a strength training program than those who consumed a placebo. This adds significant evidence to suggest that creatine monohydrate is an effective ergogenic aid to female athletes as well as males. However, the degree to which each muscle is affected needs further consideration: this study showed that upper body strength was improved, while lower body strength remained unaffected by the supplement. The relationship between creatine and body composition also requires additional research to understand if and how creatine interacts with aspects of metabolism. Furthermore, the results show that chronic creatine supplementation had no detrimental effect on blood metabolites, however, every creatine user should be aware that no study has investigated blood parameters longer than several weeks. The fact that most athletes using creatine today for extended periods of time (years) exposes the lack of information in the literature which supports or refutes the benefits of creatine as a long-term supplement.

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Table 1

TEST SCHEDULE

DAY	1	2	3	4	5	6	7	8	9	10	11	19	44	45	46
1RM Tests			X												X
Biodex	X	X											X or X		
Lactate		X											X or X		
Body water	X												X or X		
Body weight	X	X											X or X		
Bodyfat HD*, Bodyfat SF**	X												X or X		
Blood Draw	X											X			X
20g/day creatine or placebo				X	X	X	X	X	X	X					
2g/day creatine or placebo											X	X	X	X	X

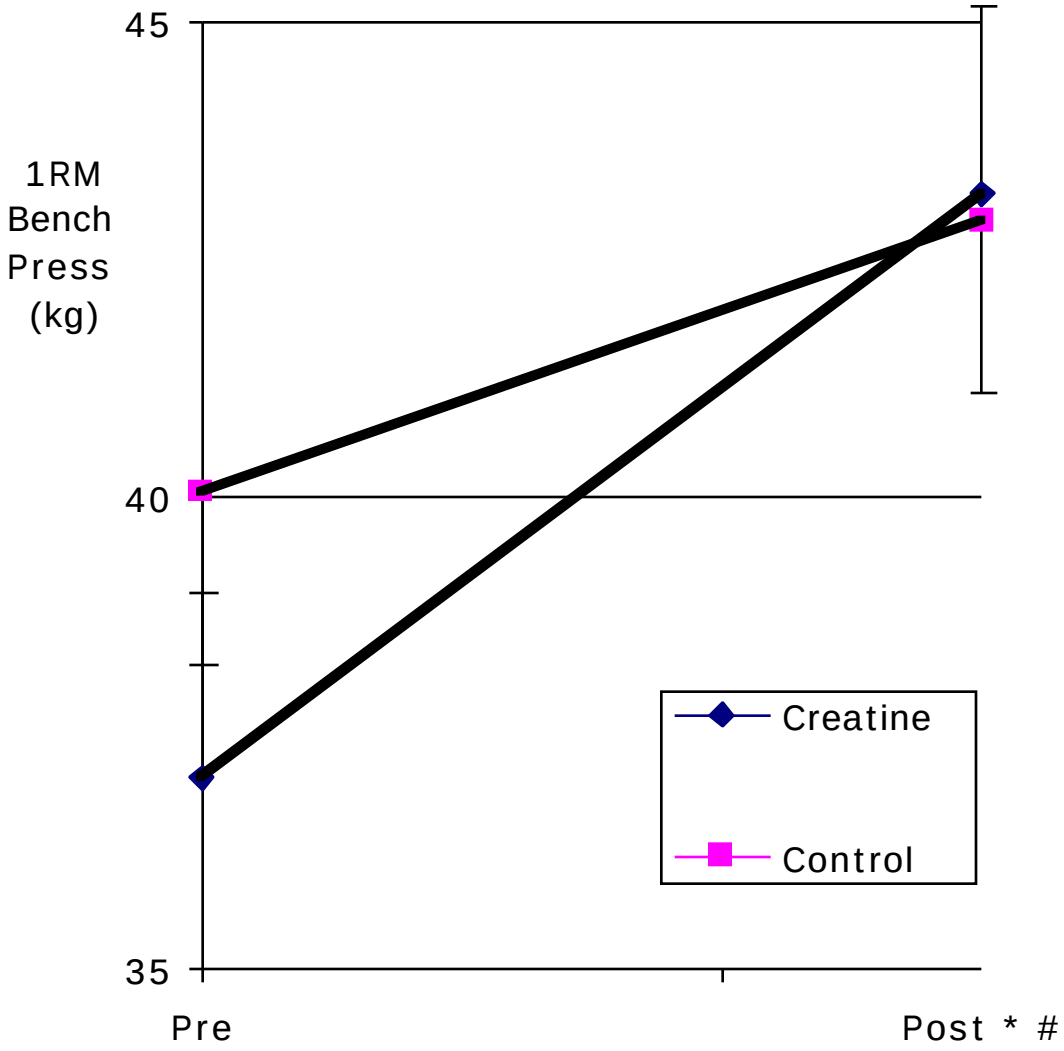
*HD=hydrodensity technique
**SF=skinfold technique

Table 2
Descriptive Statistics of Subjects

	Age (yrs.)	Height (cm)	Weight (kg)
Creatine	18.1 (1.7)	163.9 (0.8)	60.6 (2.6)
Control	19.5 (1.9)	166.2 (0.7)	61.1 (2.3)

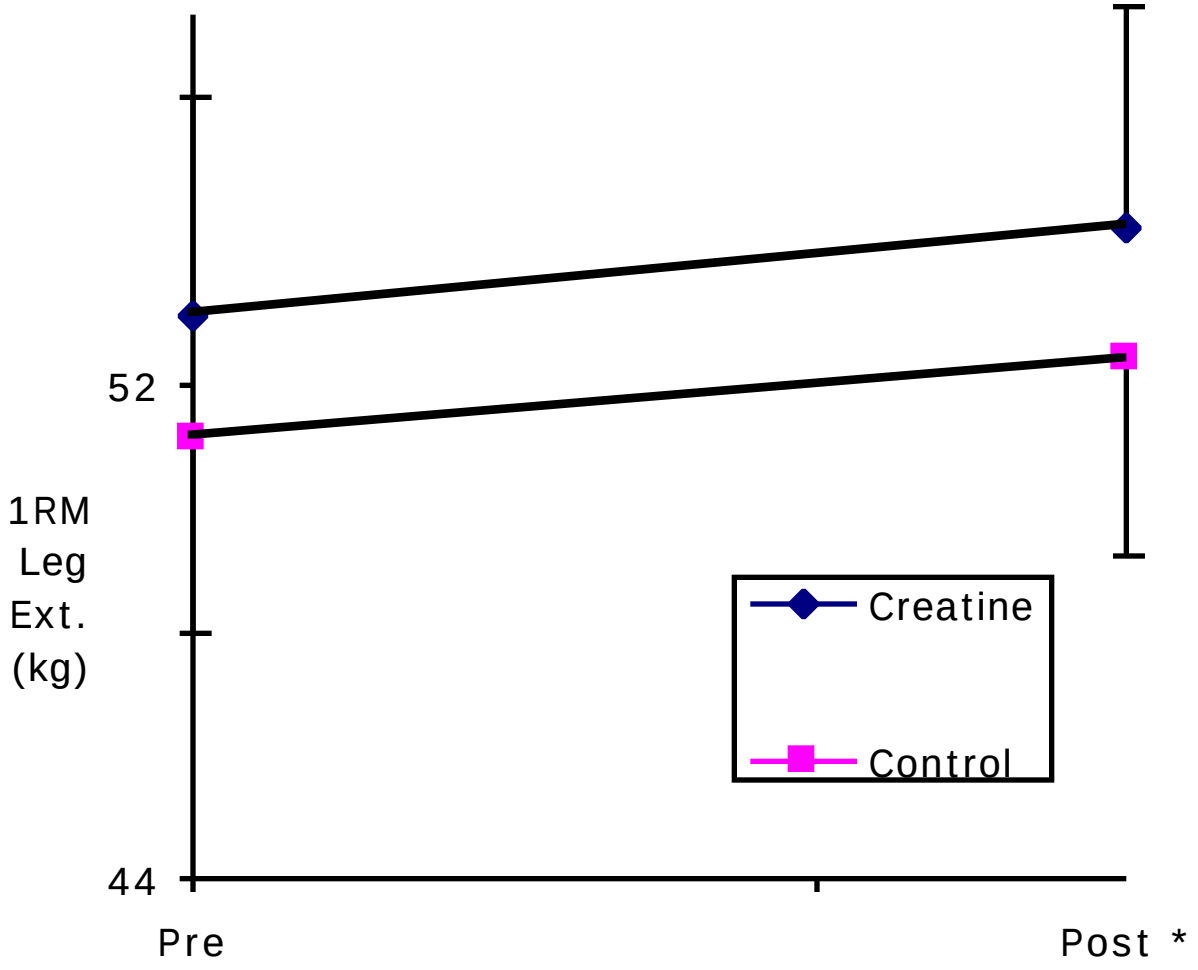
	Lacrosse Experience (yrs.)	Strength Training Experience (months)
Creatine	4.0 (2.3)	12.9 (3.2)
Control	3.8 (2.2)	13.1 (3.4)

Values are means with standard errors in parentheses.



* Significant effect of time across groups (p=<.001)
# Significant interaction of group and time (p=0.028)

Figure 1.Mean 1RM bench press values for creatine and control groups before and after the experimental period.



* Significant effect of time across groups (p=0.014)

Figure 2. Mean 1RM leg extension values for creatine and control groups before and after the experimental period.

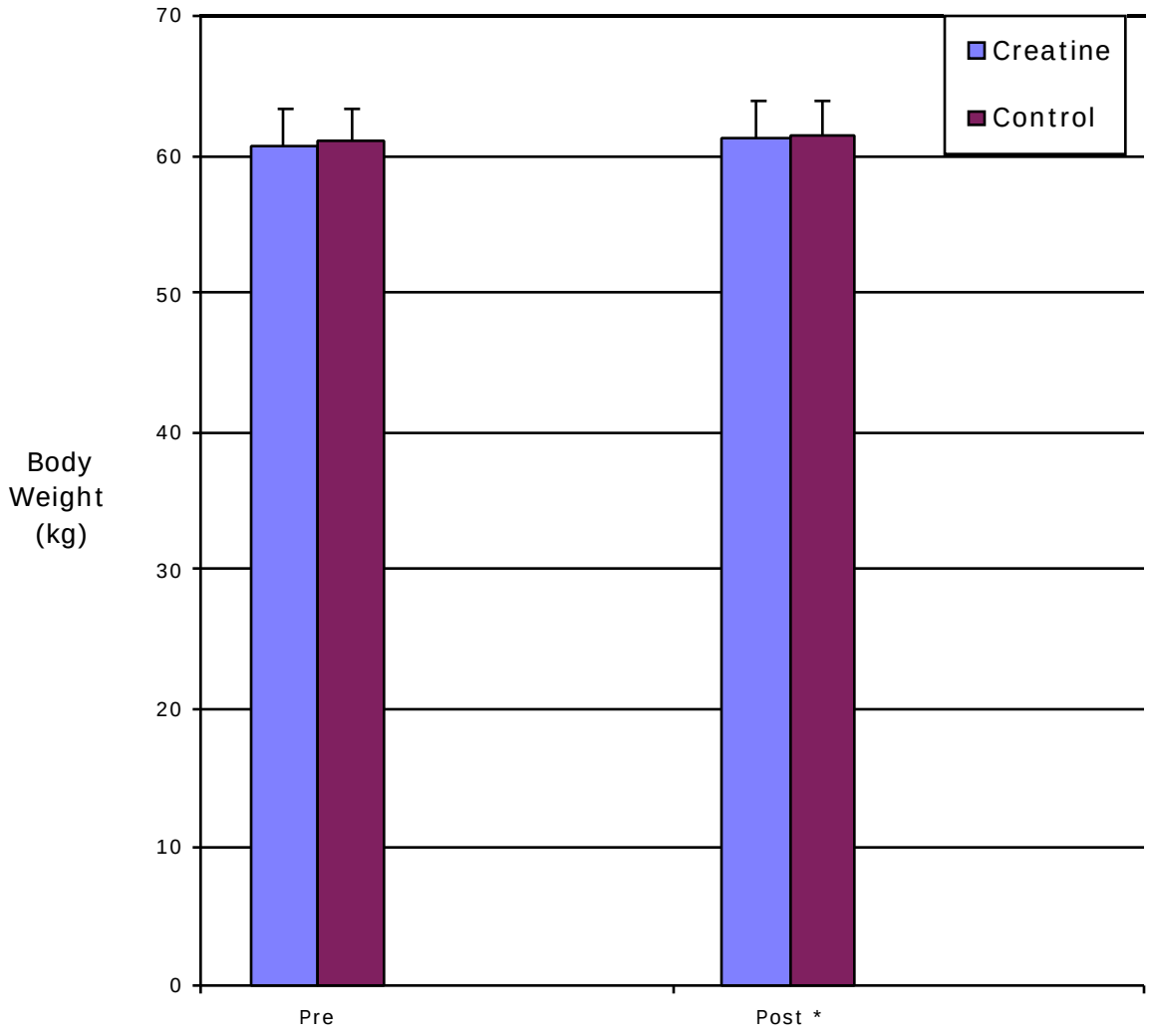
TABLE 3

Mean Total Work (TW) and Work Fatigue (WF) values for creatine and control groups before and after the experimental period.

	WF* Pre (%)	WF Post (%)	TW Pre (ftlbs.)	TW Post# (ftlbs.)
CREATINE	**149**	**145**	**5964.87**	**6366.09**
n=7	(3.08)	(3.37)	(401.15)	(401.15)
CONTROL	**146**	**157**	**6231.38**	**6397.40**
n=9	(3.15)	(3.23)	(353.78)	(352.78)

Significant time effect across groups (p=0.037)
* Work Fatigue = work fatigue of bout 5 as a percent of work fatigue of bout 1

Values are means with standard errors in parentheses.



* Significant time effect combining groups (p=0.052)

Figure 3. Mean values for body weight for creatine and control groups before and after the experimental period.

Table 4

Mean values for body composition using skinfold, hydrostatic weighing, and BIA techniques before and after experimental period

SKINFOLD TECHNIQUE

	%Bodyfat[2]		Fat-free mass (kg)	
	Pre	Post[1]	Pre	Post[1]
Creatine (n=7)	22.5 (0.92)	21.3 (0.92)	46.99 (1.92)	48.09 (1.92)
Control (n=9)	22.5 (0.18)	22.6 (0.18)	47.23 (1.69)	47.49 (1.69)

HYDROSTATIC WEIGHING

	%Bodyfat[3]		Fat-free mass (kg)	
	Pre	Post	Pre	Post
Creatine (n=7)	23.0 (1.03)	22.4 (1.03)	46.34 (1.24)	46.96 (1.24)
Control (n=9)	23.2 (0.91)	23.1 (0.91)	47.12 (1.12)	47.38 (1.12)

BIOELECTRIC IMPEDENCE (BIA)

	%Water[2]		%Body Fat	
	Pre	Post[1]	Pre	Post
Creatine (n=7)	50.86 (1.06)	47.86 (1.26)	31.71 (1.34)	35.0 (1.59)
Control (n=9)	52.11 (0.95)	51.11 (0.92)	28.89 (1.54)	30.56 (1.34)

Values are means with standard error in parentheses

1. Significant time effect across groups
2. Significant interaction between groups over time
3. Trend of time across groups (p=0.112)

Table 5

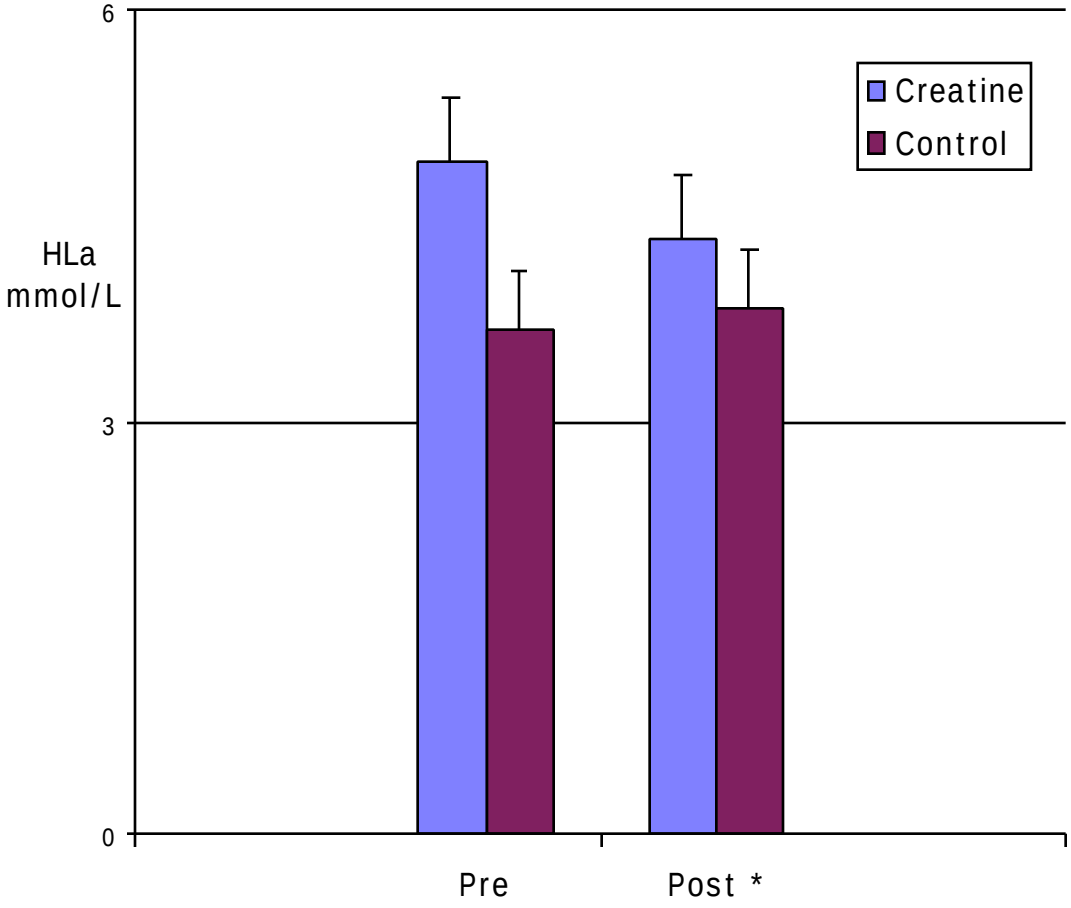
Mean GPT and BUN values for creatine and control groups
before and after experimental period

	GPT pre mmol/L	**GPT mid** mmol/L	**GPT post** mmol/L
Creatine n=7	18.81 (1.95)	17.92 (1.95)	18.59 (1.95)
Control n=9	21.29 (1.72)	21.04 (1.72)	21.80 (1.72)

	BUN pre mmol/L	**BUN mid** mmol/L	**BUN post*** mmol/L
Creatine n=7	1.25 (0.03)	1.22 (0.03)	1.30 (0.03)
Control n=9	1.20 (0.03)	1.23 (0.03)	1.29 (0.03)

***** Significant time effect across groups p=0.038

Values are means with standard errors in parentheses



* Trend for effect of group (p=0.067)

Figure 4. Changes in blood lactate accumulation in creatine and control groups before and after the experimental period.

Table 6

Blood lactate means and accumulation for creatine and control groups before and after experimental period

	BLA Pre mmol/L	**BLA Post *** mmol/L
Creatine (n=7)	4.90 (0.48)	4.33 (0.48)
Control (n=9)	3.69 (0.42)	3.82 (0.42)

	Hla pre1 mmol/L	**Hla post1** mmol/L	**Hla pre2** mmol/L	**Hla post2** mmol/L
Creatine (n=7)	1.35 (0.27)	6.25 (0.71)	1.21 (0.16)	5.54 (0.51)
Control (n=9)	1.12 (0.24)	4.82 (0.37)	1.15 (0.14)	4.85 (0.28)

BLA = blood lactate accumulation
Hla pre/post = blood lactate level before (pre) or after (post) isokinetic test, before (1) or after (2) experimental period

***** Group effect trend (p=0.067)

Values are means and standard errors in parentheses

CHAPTER IV SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH

SUMMARY

The major findings of this experiment are that a group of female athletes increased their bench press strength and decreased their percent body fat as measured by skinfold technique and percent body water as assessed by BIA, more over 5 weeks after a regimen of oral creatine supplementation than those doing the same resistance exercise program and taking a placebo. Although an increase in muscle creatine was not validated, the dose used has been previously shown to increase muscle Pcr and Cr content in men (Harris et al. 1993). The results are seemingly more significant given that the subject sample size was small (n=16).

Muscular Strength

The significant increase in strength in both groups over time suggests the efficacy of the strength training protocol. The upper body strength increase in the treatment group is consistent with several studies which report similar gains (Earnest et al.1995, Becque et al.1997 abstract). Similar to the present study, both the previous investigations involved highly-trained individuals who ingested a similar dose of creatine (20-30 g/d) for 4-6 weeks and were evaluated on 1RM, upper body strength measures. However, both of the other studies used male subjects while our data was from female subjects.

The literature reports several studies of acute supplementation having no significant effect on lower-body, high-intensity exercise as measured by treadmill running or cycle ergometry (Balsom et al.1993, Febbraio et al.1996, Odland et al.1994, and Redondo et al.1995). The common aspect of these studies is the activity level of the subjects: all were highly trained or collegiate athletes engaged in regular training programs, also similar to our study. The recent literature reports that longer term supplementation of highly-trained subjects may not result in improved strength, thus, it may be necessary for highly trained individuals to supplement for greater periods of time before significant gains are seen in high-intensity performance parameters.

The reason for an increase in upper body strength seen in the present study is difficult to explain as lower body strength did not increase as a direct result of creatine. This may be due to the fact that females are relatively weaker in their upper bodies in comparison to their lower bodies. Thus, there may have been more potential for increased creatine levels in the upper body muscles, as those muscles are less developed in female lacrosse players. It has also been shown by one group of researchers that females have higher levels of quadricep muscle creatine per tissue than males (Forsberg et al. 1991), thus creatine supplementation may have been unable to significantly augment the intially high levels of creatine in the quadriceps muscles. It is also possible that the previous physical conditioning of the subjects brought them near their maximum potential in lower body strength and therefore were unaffected by creatine supplementation.

Unpublished data has suggested that highly-trained subjects were not able to improve exercise performance with short-term supplementation. This may be a result of the already maximal activity level of the subjects. In the present study, leg extension 1RM was not affected by creatine perhaps for the same reason. If the study was longer than 5 weeks in length, supplementation may have improved performance in the lower body. Although the subjects in

this study were highly-trained, the 1RM bench press may have improved because their upper body strength may not have been as maximal as their lower body strength.

An explanation may be offered for the significant strength changes seen in the present study. Throughout the 5 week training period, the Pcr and Cr stores may have been elevated significantly, as suggested by Harris et al. (1993). The ability to resynthesize Pcr in the muscle may be greater with higher total muscle creatine. During each strength training session, the recovery of Pcr between sets of exercises may have been facilitated. This may have allowed higher work output in repeated bouts of exercise by achieving greater anaerobic ATP production, as suggested by Greenhaff et al. (1994). Thus, the increased Pcr stores may have allowed more total work to be done each session. As no significant weight gain was noted, energy during the progressively more intense training sessions may have been facilitated by bodyfat (which decreased significantly in the creatine group). The increased stimulus on the muscles may have resulted in greater strength increases over time because of increased protein synthesis. Although no direct test was performed to measure synthesis, the lower BUN values of the creatine subjects are suggestive of protein anabolism.

Isokinetic Muscular Performance

The lack of effect of creatine on the muscle performance test used in the present study are in conflict with those of Greenhaff et al.(1993) who used the same isokinetic protocol. Greenhaff and colleages reported that total muscle peak torque production during bouts 2, 3, and 4 of 30 maximal voluntary knee extensions at a constant angular velocity of 180 degrees/s, interspersed with 60 s recovery periods, was enhanced following creatine supplementation (20g/day) for 5 d in 12 moderately active, male and female subjects.

High-intensity, lower body exercise has also been examined by several researchers. Similar to the present study, however, studies which have used highly trained subjects consistently show no improvements in lower-body, high-intensity exercise performance (Balsom et al.1995, Febbraio et al.1996, Odland et al.1994, and Redondo et al.1995). However, these studies only investigated the effects of 5-7 d of supplementation. No studies have examined how long term supplementation affects high intensity, lower body, isokinetic exercise.

Body Composition

The significant gain in body weight across groups was due to the strength training. Only one published study and several studies published only as abstracts have investigated body composition changes and/or body composition changes with chronic creatine supplementation. Many authors hypothesize that the increase seen in body weight in some studies is due to an increase in body. Our study suggests that body water was not responsible for the insignificant body weight increases in our subjects.

Percent body fat decreased and fat-free mass increased as measured by skinfold technique in both groups, most likely due to the strength training program. In addition, the creatine group significantly reduced their body fat by an average of1.2%, while the control group increased their average bodyfat by 0.29%. However, no significant changes were noted when comparing creatine to control groups in fat-free mass by skinfold.

Greater pre-exercise Pcr levels and resynthesis between exercise bouts may have led to increased protein synthesis, greater muscle mass, and thus metabolic rate. Although diet

analysis was not done, if diet remained the same, an increase in metabolic rate would increase reliance on bodyfat for energy.

Blood Metabolites

Blood metabolites were monitored throughout the experimental period in order to determine the effects of large doses of creatine on kidney and liver function. Although no side effects of creatine are reported in the literature, only one study (Harris et al.1993) reported taking similar precautions and found no changes in blood profiles.

No changes were seen for blood GPT values throughout this study (Figure) and all subjects remained within the range of expected values. The trend ($p=0.062$) seen for increased BUN at the end of the study (Figure) may be a result of dehydration, which is supported by our body water data. The BUN values slightly below the expected range of 1.78-6.07 mmol/L could be due to dehydration, which is supported by our body water data, or by increased protein anabolism.

The lack of effect of time or interaction of groups over time ($p=0.067$) (Table) suggests that no benefit was seen from the strength training or the creatine in relation to blood lactate. Several other short-term studies have found that lactate levels are not altered with creatine supplementation.

Thus, higher work output (combined with no change or a decrease in blood lactate accumulation) suggests the efficacy of creatine in increasing reliance on Pcr for ATP regeneration. Although total work for both groups increased over time, no concurrent improvements in isokinetic exercise performance were noted between creatine and control groups in the present study, thus, it does not support the hypothesis that creatine favourably affects blood lactate. Further research needs to determine the role of creatine supplementation on anaerobic glycolysis during exercise.

An explanation may be offered for the significant strength changes seen in the present study. Throughout the 5 week training period, the Pcr and Cr stores may have been elevated significantly, as suggested by Harris et al. (1993). The ability to resynthesize Pcr in the muscle may be greater with higher total muscle creatine. During each strength training session, the recovery of Pcr between sets of exercises may have been facilitated. This may have allowed higher work output in repeated bouts of exercise by achieving greater anaerobic ATP production, as suggested by Greenhaff et al. (1994). Thus, the increased Pcr stores may have allowed more total work to be done each session. As no significant weight gain was noted, energy during the progressively more intense training sessions may have been facilitated by bodyfat (which decreased significantly in the creatine group). The increased stimulus on the muscles may have resulted in greater strength increases over time because of increased protein synthesis. Although no direct test was performed to measure synthesis, the lower BUN values of the creatine subjects are suggestive of protein anabolism.

In summary, the present experiment demonstrates that a regimen of dietary creatine supplementation designed to increase total muscle Cr content, significantly improved 1RM bench press strength and decreased percent bodyfat by skinfold in female athletes engaged in a strength training program. Creatine supplementation also significantly improved the 1RM bench press strength and percent body fat by skinfold more in supplemented subjects than in control

subjects. Furthermore, chronic creatine supplementation had no detrimental effect on blood metabolites.

RECOMMENDATIONS FOR FUTURE RESEARCH

Recent literature currently provides conflicting evidence regarding the benefits of creatine supplementation. Although there is an increasing amount of research currently being performed and/or published regarding creatine, the exact mechanism by which creatine ingestion enhances muscle performance is not yet clear. In addition, the topic of gender differences has yet to be further investigated. The following contains suggestions for future research:

1. Subject selection and grouping is an important task. The small sample size of this study may be a source for the insignificant statistical findings of some measures. Although subject drop-outs are unplanned, the unequal number of subjects in the test groups may have also made statistical evaluation more difficult. The subjects were also randomly grouped which resulted in insignificant, but different pre-test means. Grouping subjects based on initial data would result in similar pre-test means and easier analysis.
2. Creatine was seen to enhance most dramatically in those consuming a vegetarian diet (Delanghe et al., 1989), common in female athletes. It is likely that a population of female athletes who are historically more conscious of their body composition, and thus undergo frequent energy restriction, may benefit greatly from creatine supplementation. Creatine may not be sufficiently supplied by their non-carnivorous and/or low calorie diet. Although this type of sample selection has less validity outside the particular group of athletes, studies of this nature would benefit the individuals who may look to creatine as an ergogenic aid.
3. As creatine may be acquired by eating meats and fish, it is important to analyze the dietary habits of subjects throughout a supplementation study, in order to ensure that any intended increase in muscle creatine is a result of the supplements rather than the diet. Furthermore, when body composition measures are under investigation, diet records are equally important in the assessment of any weight fluctuation.
4. The role of creatine supplementation on anaerobic glycolysis during exercise needs to be further investigated. The results from this study do not support the hypothesis that creatine favourably affects blood lactate, an accepted marker of energy source during high-intensity exercise.
5. Future research needs to include studies using highly-trained subjects in regular resistance training programs.

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APPENDIX A METHODOLOGY

SUBJECT SELECTION AND SCREENING

The trained subjects were 20 NCAA Div.1 female lacrosse players between the ages of 18-22 who have been medically cleared by a team physician. At the time of this study (October-November 1996), the subjects were in off-season training. Subjects were excluded if they had taken creatine supplements within the last two months. This subject pool was chosen since they are required to be involved in a common resistance training program over 5 weeks, and they were likely to be highly motivated to improve performance and comply with study guidelines. Subjects were randomly distributed between two groups by the secondary investigator (advisor). The study was double blind. Permission was obtained for this study by the IRB.

Each subject completed a health questionnaire (APPENDIX C) requesting information about dietary habits (particularly vegetarianism and dieting for weight loss), body weight history, and general health . An informed consent form (APPENDIX D) was signed before the beginning of the study. Each subject was tested at the start and end of 5 weeks (Table 6) of a prescribed resistance training protocol (APPENDIX E) using the following dependent measurements: body weight, body fat and lean mass, body water, muscle strength, work fatigue, total work, and blood lactate changes following a muscle endurance bout.

BODY WEIGHT: This was assessed to the nearest 0.1 kg on a scale (Detecto Medic, Detecto Scales Inc., Brooklyn, NY) on day 1 and 2, and again on day 45 at the same time of day after an overnight fast on all three days. The subjects wore only 2-piece swimsuits on the scale.

BODY FAT AND LEAN MASS: hydrodensitometry (Novel Products) with a load cell was used to determine each subjects underwater weight on day 1 and 45. The apparatus was calibrated each day before the measurements took place. The subjects wore only 2-piece swimsuits in the hydrostatic tank and showered thoroughly before entering the tank. After initial submersion, the subjects removed excess air bubbles from their bodies. The subjects were allowed 3 practice trials before data collection took place. After slowly exhaling maximally underwater, they remained underwater for a few seconds until the measurements were taken and the values appeared on the computer screen, at which time the investigator knocked on the side of the tank to indicate the subject could rise.

The three highest values (indicating maximal air exhalation) from eight measurements were averaged and inserted into the following equation (ref) to calculate body density:

$$BD = \frac{Ma}{[Ma - Mw/Dw] - RV}$$

where
BD= body density
Ma= weight of subject in air (kg)
Mw= weight of subject in water (kg)
Dw= density of water
RV= residual volume

The density of water was determined by the temperature of the tank water matched with the corresponding density (Weast, 1967). The body density (BD) value was then inserted into Siri's equation to calculate body fat:

$$(4.95/BD - 4.5) \times 100\%$$

Residual volume of the lungs (amount of oxygen left in the lungs after forced expiration) was measured on subjects on day 45. An anesthesia bag was filled with 5L of air using a Hans Rudolph 3L syringe. Once the bag was filled, calipers were used to determine the dimensions of the bag at 5L. After calibrating the gas analyzer (Applied Electrochemistry Inc., Sunnyvale, CA), the bag was filled with 100% O2, analyzed, and recorded. The subject placed a mouthpiece in their mouth and wore a nose clip, and breathed room air normally for several minutes in order to become accustomed to the apparatus. The subject rose a finger when she had exhaled maximally. At this point, the subject took 5 deep respirations from the O2 bag. After this, the subject rose her finger to indicate complete expiration on the 5th breath. This procedure was repeated 3 times and the values were averaged. The sample from the bag was analyzed for O2, CO2, and nitrogen percentage was calculated. The residual volume was calculated from the following formula by Lundsgard and Van Slyke (1918):

$$RV = VO2 \ (b - a) / (i - b)$$

where
RV= residual volume
VO2= initial volume of oxygen in the bag
a= percent nitrogen impurity in the original oxygen
b= percent nitrogen of mixed air in the bag at the point of equilibrium
i= percent nitrogen in the alveolar air at the beginning of the experiment (standard=79.03)

Skinfold measurements were taken on day 1 and 45 at the suprailiac, thigh, and tricep sites. The subjects wore 2-piece swimsuits for this procedure. An average of three measurements were used for each site and inserted in the equation by Jackson et al. (1980) to calculate body density (BD) which is then used in Siri's equation to calculate %body fat:

$$BD = 1.0994921 - 0.0009929 (X1) + 0.0000023(X1)2 - 0.0001392(X2)$$

where
BD= body density
X1= sum of triceps, thigh, and suprailium
X2= age

BODY WATER: the RJL 101A-BIA bioelectric impedence machine was used to analyze body water at the same time of day on day 1 and day 44 after an overnight fast. The subjects wore swimsuits and laid on a cot. Alcohol swabs were used to clean the four sites of electrode attachments (2 on the right hand and 2 on the dorsal side of the right foot). The limbs of the subjects were placed in position as not to be touching any parts of the body. The electrodes were

attached to the subjects and they were instructed to remain relaxed and motionless while the machine recorded the reactance and resistance of the electrical current. Percent body fat and percent body water was calculated using the software provided by RJL.

MUSCLE STRENGTH: A 1RM free weight bench press (Nautilus) and leg extension (Nautilus) test was used to determine muscle strength at the same time of day on day 3 and day 46. The subjects warmed-up by performing 2 sets of 5 reps at 50% of their maximum weight, stretched thoroughly, and completed the tests as they are accustomed to performing 1RM tests. The subjects began the test at 85% of their previous 1RM, however, an average of three attempts was needed for each subject before their 1 RM was reached. They were allowed 3 minutes in order to rest between trials. Criteria for a full repetition on the leg extension included proper technique and a body angle such that the upper leg (thigh) was 90 degrees from the floor. Criteria for a full repetition on the bench press included proper technique (hips are never lifted off the flat bench), and full arm extension.

WORK FATIGUE and TOTAL WORK: A test of 5 X 30 maximal voluntary unilateral knee extensions (Biodex isokinetic dynamometer, Biodex Corporation, Shirley, NY) at a constant angular velocity of 180/sec, separated by a 1 min. recovery period were performed on day 1 (familiarization and orientation only) and day 2 (prior to ingestion of the first creatine supplement) and day 45 at the same time of day, 24 hours after the muscle strength tests, and after an overnight fast. The dynamomometer was calibrated once before the pre-tests and once before the post-tests. The warm-up included walking in place and stretching for several minutes. The subjects sat in the Biodex chair and their preferred leg was attached securely to the measurement arm of the Biodex machine. While they performed the repetitions, they received verbal encouragement to perform as best as they could. Work fatigue is defined as the work output in the last third of a bout subtracted from the work output in first third of the same bout, divided by the work output in the first third of a bout, and was assessed for each of the five sets. The dependent measure of work fatigue in this study was defined as the work fatigue in the last bout as a percent of the work fatigue in the first bout. Total work is defined as the total work output for each set of 30 repetitions, and was the sum of the total work done across the five bouts.

BLOOD METABOLITES: Blood lactate (YSI Model 1500 Sidekick analyzer, Yellow Springs, OH), was obtained on day 2 and day 45 from fingerprick blood samples at rest and 3 minutes after the fifth exercise bout. The lactate analyzer was calibrated each day before testing. The subject's index finger was cleaned and punctured, after which two capillary tubes were filled with blood and analyzed. The average of the two values was taken as the lactate value for that time. Blood lactate accumulation was calculated by subtracting the initial from the final concentration.

Resting blood GPT (colorimetric, Sigma diagnostics #545) and blood urea nitrogen (colorimetric, Sigma diagnostics #66) were taken from venous blood samples collected on day 3, day 19, and day 46 (after an overnight fast) to assess liver and kidney function. A certified medical laboratory technician from the Department of Human Nutrition and Foods took a blood sample from each subject's arm. The samples were centrifuged to obtain serum samples, labeled and frozen at -20 degrees Celcius until analysis. The BUN assay is for the quantitative, enzymatic determination of urea nitrogen concentration in serum at 340nm. The BUN reagent

was prepared according to manufacturer's instructions. The spectrophotometer (make and model) was set at 340nm using distilled water as a reference. Two cuvets were labelled (for reagent and sample), and 1.0mL of BUN reagent was pipetted into each. Deionized water (0.005mL) and sample (0.005mL) were added into the corresponding cuvets which were quickly mixed by gentle inversion. After 5 minutes, the absorbance was read and recorded. Triplicate copies of each sample were analyzed. The GPT assay is designed for enzymatic determination of GPT in serum. The GPT reagent was prepared according to the manufacturer's instructions. Each serum sample (0.05ml) was added to 5uL of GPT reagent, mixed gently by inversion, and placed in the spectrophotometer, calibrated and set at 405 nm. The absorbance was recorded after minute 1,2, and 3. Triplicate copies of each sample were analyzed.

The study was double-blind in that one group received 4*5g/day of creatine monohydrate (form of dietary creatine produced and donated for this study by SportPharma Inc.) for 7 days (day 4 to day 10), with 2g/day for the remainder of the study (day 11 to day 46), while the other group received a placebo (sucrose) of similar appearance for the same time period. The placebo was obtained by the manual preparation of capsules at a local pharmacy. The subjects were instructed to ingest the creatine with plenty of water, and at least 3 hours apart during the loading phase. During the maintenance phase, the subjects were instructed to ingest the capsules at the same time of day for the remainder of the study. Each subject was given a supplement log (blank calendar) in order to record the time of ingestion each day. The subjects were given the amount required for the loading period prior to the ingestion period, and they were given the remainder of the capsules after one week of loading. Throughout the supplementation period, the subjects continued their regular exercise training protocol (APPENDIX E) which consisted of a free weight bench press and Nautilus leg extension cycle based on each subject's 1RM values (ie: week 1 would consist of 4 sets of 10 repetitions at 40-60% of 1RM). In addition, the subjects complemented their resistance training with other free weight exercises such as bicep curls (standing, barbell raised to shoulder), wrist curls (hand goes through 180 degree motion with barbell), jump shrugs (standing shoulder shrugs with bar), and medicine ball throws (ball thrown from chest outward) using the maximum loads in which they were able to perform the prescribed sets and repetitions (2-6 sets of 5-10 repetitions). The exercise prescription and training was the same for all subjects, which required them to train on Monday, Wednesday, and Friday of each week.

Compliance for inclusion in this study was based on accurate ingestion of the prescribed creatine or placebo, attendance and adherence for all testing procedures, and a minimum of 85% attendance at the required strength training sessions. A sign-in sheet in the Weight Room was kept in order to monitor attendance. At the end of the 5 week period, a post-test of all dependent measures were conducted. All 1RM bench press and leg extension testing was performed in the Virginia Tech Weight Room, 130 Jamerson Athletic Center, and blood lactate, muscular endurance, body water, body weight, bodyfat, and body weight were performed in the Muscular Function Lab, 230 War Memorial Hall. Venous blood samples were obtained in 230 Wallace Hall and analyzed in 236 Wallace Hall.

The study began with 20 subjects. However, 2 subjects were dismissed from the study because of illness which caused them to miss more than the allotted weight training absences. Another subject became injured and her strength training program was modified. The final

subject who dropped out of the study did so for personal reasons. Therefore, the final number of subjects in each group was 7 in the creatine group, and 9 in the control group.

DATA ANALYSIS

A two-way ANOVA with repeated measures was used to determine differences between the placebo and treatment groups for the following dependent measures: 1RM bench press, 1RM leg extension, total work, work fatigue, blood lactate accumulation, body weight, fat-free mass by skinfold and hydrostatic techniques, body water by BIA, and percent body fat by skinfold, BIA, and hydrostatic weighing techniques. A two-way ANOVA (2X3) was used for blood metabolites GPT and BUN which were collected pre-, mid-, and post-test. Initial differences between treatment and control groups were assessed concurrent with the two-way ANOVA determinations. Correlation between pre- and post- percent body fat by skinfold and pre-and post- percent body fat by hydrostatic weighing was done to assess validity of procedures.

Statistical analysis was performed on SPSS. Correlation between pre-percent body fat by skinfold and pre-percent body fat by hydrostatic weighing was done using Minitab Statistical Analysis. Throughout the data analyses, the significance was set at an alpha level of 0.05 in order to reject the null hypotheses.

EXTERNAL VALIDITY

The characteristics of the subjects, asymptomatic, apparently healthy, college-aged female lacrosse players, allow the experimental findings from this investigation to be generalized only to the population possessing comparable characteristics.

INTERNAL VALIDITY

Variance of the measurements was minimized by: 1. familiarizing subjects with testing and training protocols and procedures, 2. conducting all testing at the same time of day, 3. limiting the participant's physical activity 12 hours before testing, 4. all testing was conducted by the principle investigator.

APPENDIX B STATISTICAL PROCEDURES

STATISTICAL PROCEDURES

A two-way ANOVA with repeated measures was used to determine differences between the placebo and treatment groups for the following dependent measures: 1RM bench press, 1RM leg extension, total work, work fatigue, blood lactate accumulation, body weight, fat-free mass by skinfold and hydrostatic techniques, body water by BIA, and percent body fat by skinfold, BIA, and hydrostatic weighing techniques. A two-way ANOVA (2X3) design was used for blood metabolites GPT and BUN which were collected pre-, mid-, and post-test. Initial differences between treatment and control groups were assessed concurrent with the two-way ANOVA determinations. Correlation between pre- and post- percent body fat by skinfold and pre-and post- percent body fat by hydrostatic weighing was done to compare procedures.

Statistical analysis was performed on SPSS. Correlation between pre-percent body fat by skinfold and pre-percent body fat by hydrostatic weighing was done using Minitab Statistical Analysis. Throughout the data analyses, the significance was set at an alpha level of 0.05 in order to reject the null hypotheses.

ANOVA TABLES

1RM Bench Press

Source of Variation	DF	SS	MS	F	P
Group	1	14.19	14.19	0.280	0.606
Group (Subject)	14	659.6	50.738		
Time	1	151.398	151.35	44.96	<0.001
Group*Time	1	20.677	20.679	6.142	0.028

1RM Leg Extension

Source of Variation	DF	SS	MS	F	P
Group	1	32.984	32.982	0.184	0.675
Group (Subject)	14	2515.65	179.69		
Time	1	14.613	14.613	7.862	0.014
Group*Time	1	0.075	0.075	0.041	0.843

Total Work

Source of Variation	DF	SS	MS	F	P
Group	1	174635.51	174635.51	0.082	0.779
Group(Subject)	14	29875734.68	2133981.05		
Time	1	633434.781	633434.781	5.326	0.037
Group*Time	1	108912.3	108912.3	0.916	0.355

Work Fatigue

Source of Variation	DF	SS	MS	F	P
Group	1	5.84	5.84	0.976	0.44
Group(Subject)	14	507.33	36.29		
Time	1	4.94	4.94	7.22	0.69
Group*Time	1	6.36	6.36	3.05	0.46

Body Weight

Source of Variation	DF	SS	MS	F	P
Group	1	0.756	0.756	0.0078	0.931
Group(Subject)	14	1363.425	97.388		
Time	1	1.904	1.904	4.517	0.052
Group*Time	1	0.092	0.092	0.0219	0.647

Bodyfat by skinfold

Source of Variation	DF	SS	MS	F	P
Group	1	3.453	3.453	0.303	0.591
Group(Subject)	14	159.611	11.401		
Time	1	2.133	2.133	4.686	0.048
Group*Time	1	3.207	3.207	7.047	0.019

Bodyfat by hydrodensitometry

Source of variation	DF	SS	MS	F	P
Group	1	1.799	1.799	0.2408	0.6275
Group(Subject)	14	104.165	7.470		
Time	1	1.039	1.039	3.139	0.112
Group*Time	1	2.365	2.375	4.899	0.826

Fat-Free Mass by skinfold

Source of Variation	DF	SS	MS	F	P
Group	1	0.249	0.249	0.0049	0.945
Group(Subject)	14	714.661	51.047		
Time	1	3.681	3.681	6.237	0.026
Group*Time	1	1.355	1.355	2.296	0.152

Fat-Free Mass by hydrodensitometry

Source of Variation	DF	SS	MS	F	P
Group	1	0.398	0.389	0.0068	0.835
Group(Subject)	14	473.367	33.546		
Time	1	2.081	2.081	2.567	0.115
Group*Time	1	1.897	1.897	3.624	0.203

%Body water

Source of variation	DF	SS	MS	F	P
Group	1	40.09	40.09	2.59	0.13
Group(Subject)	14	215.49	15.39		
Time	1	31.5	31.5	18.38	0.001
Group*Time	1	7.88	7.88	4.95	0.05

BUN

Source of Variation	DF	SS	MS	F	P
Group	1	0.0033	0.033	0.299	0.593
Group(Subject)	14	0.156	0.011		
Time	2	0.048	0.024	3.681	0.038
Group*Time	2	0.0092	0.0046	0.712	0.499

GPT

Source of Variation	DF	SS	MS	F	P
Group	1	101.697	101.697	1.302	0.273
Group(Subject)	14	1093.21	78.087		
Time	1	4.558	2.279	2.449	0.105
Group*Time	1	1.225	0.613	0.658	0.526

Blood lactate accumulation

Source of Variation	DF	SS	MS	F	P
Group	1	5.796	5.796	3.641	0.0667
Group(Subject)	14	22.575	1.572		
Time	1	0.389	0.389	0.244	0.6250
Group*Time	1	0.952	0.952	0.598	0.4457

APPENDIX C HEALTH QUESTIONNAIRE

Name: Age: DOB:
Student ID:
Local address:
Local phone number:
Permanent address:
Contact in case of emergency:
Relationship: Phone:
Primary care physician: Phone:

Medical History
Please indicate any current or previous conditions you have or had

	yes	no
heart disease or any heart problem		
rheumatic fever		
respiratory disease		
circulation problems		
kidney disease or problems		
urinary problems		
reproductive problems		
musculoskeletal problems		
fainting or dizziness		
high cholesterol		
diabetes		
thyroid problems		
high blood pressure		
lactose intolerance		
allergies to local aneasthetics		
other allergies		

if "yes" to any of the above, please indicate and explain:

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

Please list all medications you are currently taking or have taken within the past week:

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

Do you ever feel faint or short of breath with exercise?

Are there any orthopedic limitations you have which may restrict your ability to perform exercise of moderate to high intensity?
if so please describe:

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

	Y	N	relationship	age at occurrence
heart attack				
heart disease				
high blood pressure				
stroke				
kidney disease				
diabetes				

Dietary habits
please list your current weight:
what would you like to weigh:
what is the least you have weighed since age 18:
what is the most you have weighed since age 18:
has your weight fluctuated more than 5 lbs. in the past year:
if yes, by how much:;
if yes, was it on purpose?

Drinking habits

during the past month, how many days did you consume alcohol:
during the past month, how many times did you have 5 or more drinks:
on average, how many glasses of the following beverages do you consume per week:

- beer
- wine
- mixed drinks
- shots
- other

Miscellaneous

do you feel faint when you have your blood drawn
do you feel faint at the sight of other people's blood

yes no

1. Have you ingested creatine supplements
   during the past 6 months?
2. Are you on any special type of diet?
   If yes, describe:
3. Are you a vegetarian?
4. Do you eat red meat?
   How often? _____
5. Are you trying to lose weight?
6. Are you taking any diet pills, laxatives, or
   other drugs in order to lose weight?
7. Has your weight fluctuated more than
   10 pounds in the past 12 months?
   2 months?
8. What is the most you have weighed since you were 18 years old?
9. What is the least you have weighed since you were 18 years old?
10. Have you used a dietary supplement in the past?

How long ago? Do you currently use one now?

APPENDIX D INFORMED CONSENT

Title of Study: The Effects of Chronic Creatine Supplementation on Performance and Body Composition in Female Athletes

Purpose of the Study: To determine if creatine supplementation affects exercise performance or body composition with concurrent strength training.

Procedures:

This study will span 5 weeks, during your resistance program assigned by your strength coach. Prior to the start of the study, you will undergo several tests which will be repeated at the end of the study at the same time of day and in the same order.

Two days prior to the supplement ingestion, you will come to Wallace Hall Rm. 328 (after an overnight fast) where a certified nurse will draw a very small sample of blood from your arm. You will then go to the Muscular Performance Lab in War Memorial Hall. Your body weight will be measured using a simple scale. Your body water will be measured using bioelectrical impedence whereby a small electrode is placed on your right hand and right foot and a harmless, low-intensity current (which cannot be felt) flows through your body.

Your body fat and lean mass will be measured using the underwater tank. You will sit on a chair immersed in the tank, and the measurements will not begin until you are completely familiar with the procedures. During this test, you will be required to exhale completely and remain submersed for a few seconds while the measurement takes place. Your residual volume (amount of air left in your lungs after forced expiration) will be measured by having you breathe 5 times into a rubber bag filled with oxygen.

The muscle work test will measure the work output of your thigh muscle. You will sit on a chair attached to the Biodex machine, which looks like a leg extension machine. Your dominant leg will be strapped down over your thigh and shin. The shin pad is attached to an arm which connects the to the measurement device in the machine. You will be asked to perform 5 sets of 30 maximal kicks with this leg with a 1 minute recovery period between sets. This type of testing is very safe since the machineís resistance adjusts to your effort. The entire testing on this day will take approximately 1.5 hours.

The next day, after a standardized meal, you will report to the Weight Room. You will warm-up and stretch before completing the 1RM tests. Once the maximum weight has been lifted, you will cool down and stretch. This procedure will be followed for both the knee extension and bench press tests. The following day is the beginning of the experimental period. You will be given creatine supplements for 4 weeks. It is important that you follow the instructions carefully, if not, you may be withdrawn from the study. It is important that you consume all the supplement we give you and that you do not consume any other supplements without checking with the investigators first. At the mid-point of the experiment (day 16), your blood will be drawn again, under the same conditions, to ensure that your liver and kidney functions are unaffected by the creatine supplements. The day after your last supplement, you will report to the Muscular Performance Lab after an overnight fast and undergo the same tests in

the same order as before. The next day, you will report to the Weight Room and perform the same 1RM tests.

Subject Responsibilities:
1. Attendance at an informational session to assess qualification to participate in study.
Upon Qualifying As A Subject, the Following Apply:
2. Subjection to the measurements of 1RM muscular strength, body composition, body weight, body water, and muscle work one time prior to the study and one time at the end of the study. Blood samples will be taken 3 times throughout the study, and diet recording will occur 2 times in 4 weeks.
3. Ingestion of 20g/day of creatine supplement for the first 6 days, and 2g/day for the remainder of the study.
4. Adhering your regular training protocol of prescribed strength exercises throughout the 4 weeks.
5. Abstinence of exercise, smoking, and alcohol 24 hours prior to all testing.
6. Fasting at least two hours prior to all testing.
Attend exercise testing sessions in the Weight Room in Jamerson Athletic Center. All other measures will be performed in the Muscular Function Laboratory in War Memorial Hall.
You must inform us of any medical conditions which may arise during the study, or of any known HIV, hepatitis, or other transmissible disease.
Risks Associated with Participation:
Temporary muscle soreness and/or strains due to the strength training and muscular endurance tests, fatigue, irregular heart rhythm, and unexpected sudden death are possible risks associated with high-intensity exercise. However, since you are familiar with the exercises, little discomfort should be experienced. The risks of blood withdrawl include minor bleeding, bruising, and/or swelling. These will be minimized by the medical technicianís certified ability and experience.
Personal Benefits Associated with Participation:
1. 1RM values
2. %body fat
Confidentiality:
I understand that any data collected will be held in confidence and will only be used for the purpose of this study. I also understand that my name will not be used in the presentation or publication of the study. The results of my tests will not be released to anyone without the written consent of the subjects.
Freedom to Withdraw:
I understand that I have the right to withdraw from this study if at any time I feel that the participation may be hazardous to my health. The researcher also has the right to terminate my participation should she feel that it may be hazardous to my health or if she feels I have not complied with the requirements of the study.
It is also the participant's responsibility to inform the researcher of any discomforts or injuries, as well as any medical problems (pre-existing or not) that may occur during the course of the study or affect the study in any way. I am aware that my blood may be tested for HIV if

an experimentor becomes exposed to my blood. In the course of any medical emergency a telephone will be available to notify emergency services.

I have read and understood the above statements and have had the opportunity to ask questions. I understand that the researcher will, at any time, answer my inquiries concerning the procedures and protocols used in this study.

Approval of Research:

This research project has been approved, as required by the Institutional Review Board for projects involving human subjects at Virginia Tech.

Subject Permission:

I have read the informed consent and fully understand the procedures and conditions of this project. All my questions have been addressed and I consent to participate in this research study.

If I participate, I may withdraw at any time without penalty. I agree to abide by all the rules of this project.

If at any time I have questions, I will contact:
Principle Investigator: Megan Brenner 540-953-3325
Project Chairperson: Dr. Janet Rankin 540-231-6355
University Institutional Review/Research Division: Tom Heard
540-231-5787

Subject's Signature:_____ Date: _____

APPENDIX E STRENGTH TRAINING PROTOCOL

BENCH PRESS AND LEG EXTENSION CYCLE

Subject pre-test 1RM bench press: _____ lbs.
Subject pre-test 1RM leg extension: _____ lbs.

WEEK 1:
Bench/Leg extension
40%=____/____ X 10
55%=____/____ X 10
60%=____/____ X 10
60%=____/____ X 10

WEEK 2:
Bench/Leg extension
40%=___/____ X 10
60%=___/____ X 10
70%=___/____ X 10

WEEK 3:
Bench/Leg extension
40%=____/____ X 10
60%=____/____ X 6
70%=____/____ X 6
75%=____/____ X 6

WEEK 4:
Bench/Leg extension
40%=___/____ X 10
70%=___/____ X 5
75%=___/____ X 5
85%=___/____ X 5
85%=___/____ X 5

WEEK 5
Bench/Leg extension
50%=____/____ X10
75%=____/____ X 5
80%=____/____ X 5
85%=____/____ X 5
85%=____/____ X 5

ADDITIONAL EXERCISES:

MONDAY	WEDNESDAY	FRIDAY
1.Jump rope (warm-up)	Jump rope (warm-up)	Jump rope (warm-up)
2. Situps (60)	Situps (60)	Situps (60)
3. Leg extension 3 sets, 8 reps (Nautilus knee extension machine) (reps=repetitions)	Leg extension 3 sets, 8 reps	Leg extension 3 sets, 8 reps
4. Jump shrugs (with bar, standing shoulder shrugs) 2 sets, 5 reps	Jump shrugs 2 sets, 5 reps	Jump shrugs 2sets, 5 reps
5. Back Squats (with bar, from standing until knee a 90 degrees) (see cycle)	Back Squats	Back Squats
6. Bench press (supine on bench with bar lifted from chest to full elbow extension) (see cycle)	Bench press	Bench press
7. Lat pulldowns 4 sets, 8 reps (Nautilus latissimus dorsi exercise)	Lat pulldowns 4 sets, 8 reps	Lat pulldowns 4 sets, 8 reps
8. Hammer curls 6 sets, 8 reps (free weight, standing, bicep curls)	Hammer curls 6 sets, 8 reps	Hammer curls 6 sets, 8 reps
9. Medicine ball throws (ball thrown from chest forward) 3 sets, 6 reps	Medicine ball throws 3 sets, 6 reps	Medicine ball throws 3 sets, 6 reps
10. Wrist curls (free weight wrist isolation exercise) 3 sets, 8 reps	Wrist curls 3 sets, 8 reps	Wrist curls 3 sets, 8 reps

APPENDIX F DATA COLLECTION SHEET

Date:_____ Subject #:_____ eat/drinkbefore?_____

Height: _____ cm **Weight**:_____ kg

H20 weight (lbs.) H20 temp:___ F Body Water
_____ _____ reactance:
_____ _____ resistance:
_____ _____ **body water=**
_____ _____
Avg.=
%body fat =

Skinfold (mm):
thigh: _____ _____ _____ _____ avg:
abd: _____ _____ _____ _____ avg:
tricep: _____ _____ _____ _____ avg:
%body fat =

Residual volume (L):
O2: _____ _____ _____ avg:
CO2: _____ _____ _____ avg:
RV =

Strength Testing 1RM
Bench Press: _____lbs. **Leg extension**: _____ lbs.

Biodex

WF pre	**WF pre**
WF post	**WF post**
TW pre	**TW pre**
TW post	**TW post**

Blood lactate
pre: _____ _____mmol/L
post : _____ _____mmol/L time post: _____
accumulation: post-pre: _____

Blood **GPT**				Blood **BUN**			
pre:	_____	_____	_____	_____	_____	_____	_____
mid:	_____	_____	_____	_____	_____	_____	_____
post:	_____	_____	_____	_____	_____	_____	_____

APPENDIX G PILOT DATA

The graph of 1RM bench press on the following page was collected using two female subjects who were tested one day (day 1) and 24 h later (day 2).

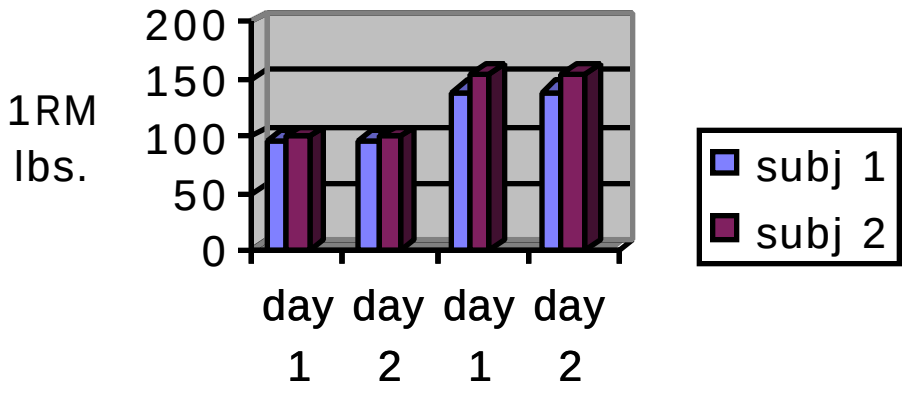
The body composition graph on the next page was collected on one subject only, to investigate test-retest measures.

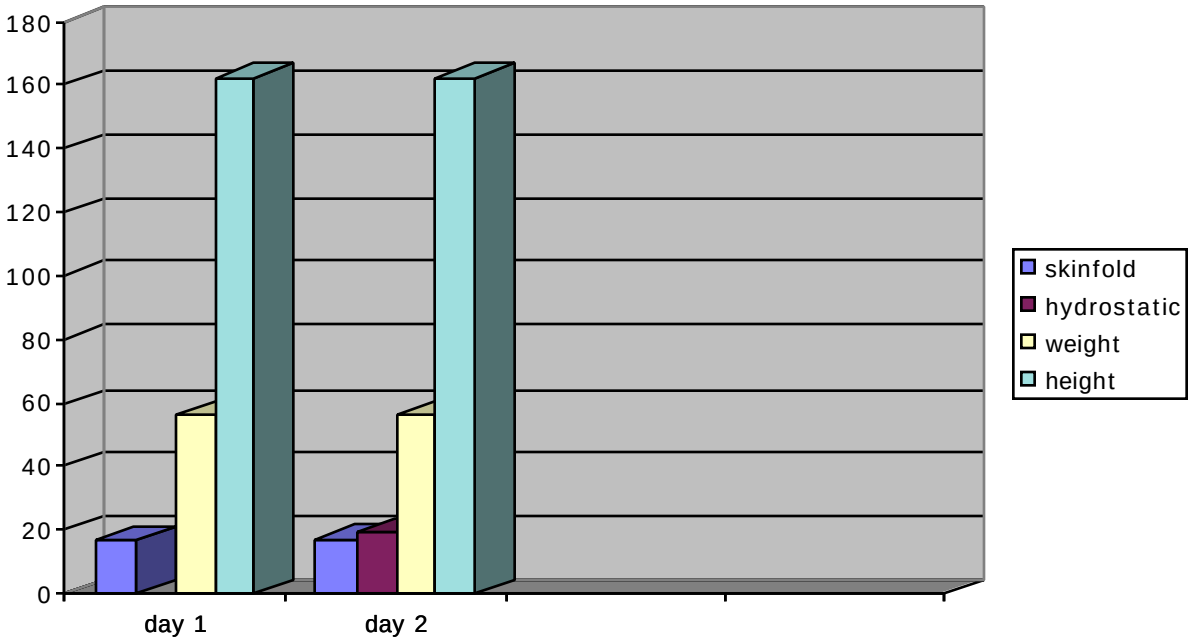
The data on the current page was taken using one subject over a two-day period to investigate test-retest measures:

Subject: pilot Age: 20

Day 1	Day 2
Height: 162 cm	162 cm
Weight: 56.25 kg	56.50 kg
H20 Temp: 86 F	90 F
H20 weight (lbs.): inaccurate methods	4.6907 4.6872 4.6398 4.6248 Avg.= **4.6610** **%body fat = 19.26**
Skinfold (mm): thigh: 19.5, 17.0, 18.25 - **18.25** abd: 8.0, 8.5, 9.0 - **8.5** tricep: 13.5, 14.0, 14.0 - **13.83** **%body fat = 16.98**	20.0, 20.25, 18.0 **- 19.41** 8.0, 9.0, 9.0 - **8.66** 13.0, 14.5, 14.0 **- 13.83** **%body fat = 17.16**
H20 reactance: 43 resistance: 720 **body water = 53%**	56 698 **body water = 57%**
Residual volume (L): N/A	O2: 85.20, 84.76, 84.23 - **84.73** CO2: .96, .89, .84 - **.896** **RV = .851**

PILOT DATA


1RM
lbs.
200
150
100
50
0
day 1
day 2
day 1
day 2
subj 1
subj 2


180
160
140
120
100
80
60
40
20
0
day 1
day 2
skinfold
hydrostatic
weight
height

APPENDIX H
IRB Proposal

TITLE OF STUDY: The Effects of Chronic Creatine Supplementation and Strength Training on Body Composition and Muscular Performance in Female Athletes

INVESTIGATORS: Megan Brenner, MS candidate
Janet Walberg Rankin, Ph.D, Faculty Advisor
Department of Human Nutrition, Foods, and Exercise

JUSTIFICATION:

The majority of creatine found in the body is located in skeletal muscle. Recent published results demonstrate that oral supplementation of 20g/day of creatine increases muscle creatine in untrained males. Currently, this dietary supplement is being tested on a variety of different subjects because of its ability to increase creatine stores in skeletal muscle, thereby possibly delaying fatigue and/or improving performance parameters in which the creatine phosphate system is the primary source of energy. It is a popular topic in the sports nutrition world, and must be further researched to determine whether it has value as an ergogenic aid or if it is a costly placebo. Today, many varsity athletes here at Virginia Tech are ingesting creatine, and many of them have believe that creatine is a key factor in their improvement. As a result of the new interest in creatine supplementation and the relatively small literature base on this subject, we plan to study the effect of this supplement on performance and body composition of female athletes while also measuring some blood factors to insure safety of longer term creatine supplementation.

Several studies have looked at the effect of creatine on performance. Creatine supplementation increased total muscle peak torque production in untrained subjects during repeated bouts of maximal knee extensions. During 10 6-second bouts of high-intensity, intermittent cycling, creatine supplementation increased work output toward the end of each exercise bout. In contrast, creatine did not increase power output during continuous, high-intensity cycling , or a single, 30-second maximal cycling bout.

In addition to improved performance, a few studies have noted changes in body composition related to creatine supplementation. Lean body mass has been found to increase by approximately 0.5 kg with concurrent acute creatine ingestion in some subjects, however, there have been no studies examining peformance changes with chronic supplementation, nor with female subjects.

Only one published study to date has examined the effects of creatine supplementation during resistance training. Thus the issue of chronic ingestion during training needs to be studied. The majority of studies examining creatine supplementation have used only male subjects. A few studies have combined male and female subjects but did not analyze effects seperately by sex. The studies available which relate to muscle creatine content and gender are also inconclusive. One study demonstrated that females

have a higher creatine store in relation to tissue weight. Overall, there is a lack of information regarding the effects of creatine supplementation on women. Creatine uptake was also seen to increase most dramatically in those consuming a vegetarian diet, common in female athletes . It is likely that a population of female athletes who are historically more conscious of their body composition and thus undergo frequent energy restriction may benefit from creatine supplementation. Creatine may not be sufficiently supplied by their non-carnivorous and/or low calorie diet. These dietary habits, coupled with the constant desire to enhance performance, make female varsity athletes attractive candidates for a creatine supplementation study.

In summary, previous research has suggested that creatine supplementation may improve anaerobic exercise performance in males. It is not clear whether there is also a benefit of this supplement for females. Most studies have been done using short-term supplementation (in spite of the fact that most athletes use it over a long-term period), but there is some evidence that there may be a positive effect of long-term supplementation with concurrent strength training. Thus, this study will examine the possible effects on performance and metabolism of chronic creatine supplementation on female athletes involved in regular resistance training.

METHODOLOGY

The trained subjects will be 30 NCAA Div.1 female lacrosse players between the ages of 18-22 who have been medically cleared by a team physician. Subjects will be excluded if they have taken creatine supplements within the last two months. This subject pool was chosen since they are required to be involved in a common resistance training program over 6 weeks, and they are likely to be highly motivated to improve performance and comply with study guidelines. The latter will be assisted since the principle investigator has been a player and coach for this team. Subjects will be distributed between two groups on the basis of initial strength per kg of body weight for back squat, and by vegetarian or carnivorous diet habits.

Each subject will be asked to complete a survey (see attached) requesting information about dietary habits (particularly vegetarianism and dieting for weight loss) and body weight history, and a health survey. An informed consent form (see attached) will be signed before the beginning of the study. Each subject will be tested at the start and end of 6 weeks of resistance training (3 times per week)using the following dependent measurements: body weight, body fat and lean mass, body water, muscle strength, muscle endurance, and blood metabolite changes with exercise.

BODY WEIGHT: This will be assessed to the nearest 0.1 kg on a scale on day 1 and 2, and again on day 45 and 46 at the same time of day after and overnight fast on all four days.

BODY FAT AND LEAN MASS: An underwater tank will be used to determine each subject's underwater weight (three highest values from eight measurements will be used). The subjects will be required to exhale completely and remain submersed for a few seconds while the measurements take place. Residual volume of the lungs (amount of

oxygen left in the lungs after forced expiration) will be measured on subjects at the same time of day on day 1 and day 44 after an overnight fast.
BODY WATER: This involves bioelectric impedence in which a harmless, low-intensity current (running from electrodes on the right foot and right hand) will analyze body water at the same time of day on day 1 and day 44 after an overnight fast.
MUSCLE STRENGTH: A 3RM free weight bench and free weight back squat test will determine muscle strength at the same time of day on day 1 and day . These measurements will be done between 2 and 5 pm, at least 2 hours after a standardized meal. The subjects will be required to warm-up and stretch completely and perform the test as they are accustomed to performing 3RM tests. Criteria for a full repetition on the back squat will include proper technique and a body angle such that the upper leg (thigh) is 90 degrees from the floor. Criteria for a full repetition on the bench press will include proper technique (hips are never lifted of the flat bench), and full arm extension.
MUSCLE ENDURANCE: A test of 3 X 30 maximal voluntary unilateral knee extensions at a constant angular velocity of 180/sec, separated by a 1 min. recovery period will be performed on day 2 (prior to ingestion of the first creatine supplement) and day 45 at the same time of day (Biodex isokinetic dynamometer), 24 hours after the muscle strength tests. The subjects will sit in a chair and their dominant leg will be attached securely to the measurement arm of the Biodex machine while they perform the repetitions.
BLOOD METABOLITES: Blood lactate (YSI Model 1500 Sidekick analyzer), plasma glucose (colorimetric), and ammonia (colorimetric) will be obtained from venous blood samples (10mL) at rest and immediately after the third exercise bout. Resting blood GPT (colorimetric) and urea nitrogen (colorimetric) will be assessed to monitor liver and kidney function. Just before the start and at the end of the exercise a certified medical laboratory technician from the Department of Human Nutrition and Foods will take a blood sample from each subject's arm.

Subjects will complete a three day diet journal of all food and beverage consumption every two weeks throughout the course of the study. This will be analyzed using Nutritionist IV to determine whether diet changes occurred. At the mid-point of the experimental period, fasted blood samples will be obtained from the subjects in order to monitor liver and kidney function. The study will be double-blind where one group will receive 4*5g/day of creatine monohydrate (form of dietary creatine produced by commercial manufacturers) for 7 days with 3g/day for the remainder of the study, while the other group will receive a placebo (glucose) of similar appearance for the same time period. The subjects will continue their regular exercise training protocol which consists of a bench and squat cycle based on each subject's predicted 1RM values. In addition, the subjects will be complementing their resistance training with other free weight exercises using the maximum loads in which they are able to perform 3 sets of 8 repetitions. Note that the exercise prescription and training will be the same for all subjects. At the end of the 6 week period, a post-test of all dependent measures will be conducted.

SAFETY:

In order to comply with OSHA regulations, gloves and laboratory gowns will be worn at all times by the researchers in contact with blood samples. A Biohazard container will be used for the disposal of all blood samples, and a Sharp's container for all needles. A certified laboratory medical technician from the Department of Human Nutrition and Foods will perform all phlebotomy procedures. All researchers will have attended an informational session on OSHA regulations.

SUPERVISION:

All testing will be performed in the Jamerson Athletic Center Weight Room and the Muscular Function Laboratory in War Memorial Hall. An SCSC strength coach will be in attendance during testing, and all those conducting the exercise testing will be certified in CPR. A telephone will be accessible to activate Advanced Health Life Support in case of an emergency. As individuals are varsity athletes, the risks of any complications are very low.

RISKS AND BENEFITS:

The risks associated with completing the above mentioned exercise evaluations include fatigue, muscle soreness, muscle strains/pulls, irregular heart rhythm, and unexpected sudden death. No side effects of creatine supplementation have been observed in other studies. However, the risks of any harmful effects will be diminished by the assessment of indicators of liver and kidney function. These risks are remote since the subjects will be trained and medically cleared by the team physician. Furthermore, most of the experiments in this study are very familiar all subjects. The risks of blood withdrawl may include some minor swelling and/or bruising. Benefits from this study include data on one's body composition and strength levels. No financial compensation will be provided.

CONFIDENTIALITY:

The data collected from this study will be kept strictly confidential. At no time will the investigators release the data to anyone other than the individuals working on the project without the subject's written consent. All data collected will be identified by subject number.

BIOGRAPHICAL SKETCH:

Megan Brenner, MS candidate: Graduate student in Exercise Science with an emphasis in Cardiac Rehabilitation. Bachelor's Degree in Chemistry from Whittier College, CA. Virginia Tech Women's Lacrosse Assistant Coach. Currently certified in CPR.

Janet Walberg Rankin, Ph.D., Faculty Advisor: Associate Professor in Human Nutrition and Foods. Dr. Rankin received 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 Exercise 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 I SUMMARY OF DEPENDENT MEASURES AND RESULTS

Abbreviations:
C= creatine group
P= placebo group

MAJOR DEPENDENT VARIABLES:

MEASURE	RESULTS (all increases $p<0.05$)
1. 1RM bench press strength	increase in C and P groups increase in C more than P
2. 1RM leg extension strength	increase in C and P groups
3. Total work	increase in C and P groups
4. Fat-free mass by hydrostatic weighing	no change
5. Fat-free mass by skinfold	increase in C and P groups
6. Body weight	increase in C and P groups
7. Percent body fat by skinfold	increase in C and P groups increase in C more than P
8. Percent body fat by hydrostatic weighing	no change

MINOR DEPENDENT MEASURES

MEASURE	RESULTS (all increases $p<0.05$)
1. Work Fatigue	no change
2. Blood lactate accumulation	no change
3. Percent body water by BIA	decrease in C and P groups decrease in C more than P
4. Percent body fat by BIA	invalid results
5. BUN	increase in C and P groups
6. GPT	no change

APPENDIX J RAW DATA

The following abbreviations and units apply to the raw data tables:

for all measures
pre/1: at start of experimental period
mid: at midpoint of experimental period
post/2: at end of experimental period
subjects 1,5,7,9,12,14,16 are in creatine group
subjects 2,3,6,10,13,15,17,18,19 are in placebo group

Subject: subject ID number
Age: age in years at start of study
Height: height in cm
BW: body weight in kg
%pre/post skin: percent body fat measured by skinfold
%pre/post H20: percent body fat measured by hydrostatic weighing
pre/mid/post GPT: GPT in mmol/L
pre/post Hla1: blood lactate value in mmol/L before isokinetic knee test
pre/post Hla2: blood lactate value in mmol/L after isokinetic knee test
acc 1/2: blood lactate accumulation (lactate value after test minus lactate value before test) in mmol/L
pre/mid/post BUN: BUN in mmol/L
TW pre/post: total work in Watts across 5 sets of isokinetic knee test
FFM S1/2: fat-free mass in kg measured by skinfold
FFM H1/2: fat-free mass in kg measured by hydrostatic weighing
Bench 1/2: 1RM bench press strength in kg
Leg 1/2: 1RM leg extension strength in kg
WF pre/post: work fatigue in percent for isokinetic knee test
(work fatigue in fifth bout as a percent of work fatigue in first bout)
Bw 1/2: percent body water measured by BIA
BIA fat1/2: percent body fat by BIA

The following data was derived from a self-described questionnaire
(APPENDIX C)
Vegetarian: subjects who considered themselves non-meat eaters
Meat: subjects who consumed red meat, poultry, and/or fish less than two times per month
Lax Exp: lacrosse experience in years
Strength Exp: strength training experience in months

Subject	Age	Height	BW Pre	BW Post
1	18	172	59.75	60
5	21	169	67.5	67
7	18	154	53.5	54
9	19	166	65.5	66
12	18	170	61	62.5
14	19	163	63.75	64
16	18	157	53.5	55.2
2	18	163	57	58.5
3	19	168	72.5	73.5
6	19	166	59.5	59.5
10	18	164	62	63.75
13	20	155	51	51
15	19	160	52.8	52.75
17	19	179	75	73.5
18	18	157	57.5	58.5
19	20	168	62.25	62

Subject	%preskin	%preH20	%postskin	%postH20
1	22.72	23.68	22.46	23.25
5	22.64	25.98	21.48	25.98
7	21.43	22.97	20.94	21.03
9	24.91	22.79	23.86	22.65
12	16.98	17.92	17.25	16.47
14	25.54	25.83	23.68	24.46
16	22.98	21.68	19.42	22.96
2	21.68	20.26	20.95	21.16
3	22.87	25.96	22.36	25.74
6	19.04	20.36	19.68	20.21
10	26.86	25.51	25.98	25.74
13	21.18	23.23	21.83	22.98
15	19.63	19.19	20.08	19.64
17	23.68	25.02	24.22	24.96
18	24.43	25.96	24.92	25.93
19	22.96	23.68	23.37	21.48

Subject	pre gpt	mid gpt	post gpt
1	17.35	15.48	16.58
5	18.79	19.89	19.89
7	19.34	19.89	19.46
9	20.99	20.995	20.99
12	20.44	18.785	19.53
14	20.44	17.685	18.23
16	14.36	12.71	15.47
2	17.13	16.025	17.67
3	20.44	19.89	19.89
6	18.347	19.253	18.96
10	19.93	16.58	19.13
13	18.35	18.92	19.21
15	22.18	21.056	22.945
17	17.13	16.269	17.173
18	22.843	22.16	22.532
19	35.36	39.16	38.68

Subject	pre HLa1	postHLa1	preHLa2	postHLa2	acc 1	acc 2
1	1.98	5.01	1.68	5.57	3.03	3.89
5	0.73	8.74	0.7	7.53	8.01	6.83
7	1.44	7.96	1.23	4.3	6.52	3.07
9	0.42	3.87	0.96	4.92	3.45	3.96
12	1.5	4.96	1.91	3.94	3.46	2.03
14	2.5	7.84	0.95	7.05	5.34	6.1
16	0.91	5.37	1.05	5.45	4.46	4.4
2	0.42	4.63	0.86	3.57	4.21	3.77
3	1.48	5.55	0.71	4.67	4.07	3.96
6	0.88	4.31	0.89	4.51	3.43	3.62
10	1.09	3.57	1.5	3.96	2.48	2.46
13	0.96	4.39	0.79	4.74	3.43	3.95
15	0.85	3.41	1.18	5.09	2.46	3.91
17	2.73	7.01	1.71	6.27	4.28	4.56
18	0.22	4.55	0.88	5.1	4.33	4.22
19	1.43	5.95	1.86	5.75	4.52	3.89

Subject	pre bun	mid bun	post bun
1	1.105	1.277	1.238
5	1.135	1.191	1.251
7	1.36	1.1915	1.402
9	1.598	1.185	1.28
12	1.137	1.285	1.196
14	1.286	1.2835	1.443
16	1.1615	1.1515	1.282
2	1.242	1.268	1.313
3	1.208	1.242	1.371
6	1.196	1.189	1.192
10	1.141	1.269	1.273
13	1.182	1.179	1.189
15	1.163	1.172	1.251
17	1.159	1.217	1.298
18	1.256	1.245	1.367
19	1.251	1.339	1.375

Subject	TW pre	TW post	WF pre	WF post
1	5490.4	6421	143	106
5	6359.7	5922.1	142	13
7	5336.1	5622.4	152	189
9	6365.3	7328.2	169	16
12	6990.9	6836.5	133	136
14	6214.2	6411.1	112	119
16	4997.5	6021.3	186	179
2	5542.9	5502.1	254	213
3	8175.3	8941.8	152	166
6	7374.2	7296.3	147	224
10	6178.1	6579.8	115	87
13	5104.2	5006.2	128	134
15	4768	5489.2	094	214
17	7961.5	7545.5	166	115
18	5438	5697.6	129	119
19	5540.3	5518.1	126	148

Subject	FFM S1	FFM S2	FFM H1	FFM H2
1	46.17	46.52	47.05	47.95
5	52.22	52.61	50.96	51.84
7	42.03	42.69	45.38	43.95
9	49.18	49.75	50.39	51.03
12	50.64	51.72	50.21	51.53
14	47.47	48.84	46.94	46.37
16	41.21	44.48	42.78	44.01
2	44.64	46.24	43.57	44.93
3	55.92	57.07	54.78	56.07
6	48.18	47.79	48.65	48.24
10	45.35	47.19	46.66	47.31
13	40.2	39.87	41.37	40.68
15	42.44	42.16	43.66	42.27
17	57.24	55.7	56.46	55.86
18	43.1	43.92	42.67	42.02
19	47.96	47.51	46.78	47.42

Subject	Bench 1	Bench 2	Leg 1	Leg 2
1	31.8	40.9	57.9	59.1
5	40.9	47.7	56.8	61.3
7	38.6	43.2	45.4	45.4
9	36.4	43.2	60.2	61.3
12	38.6	45.4	59.1	59.1
14	40.9	43.2	51.1	54.5
16	31.8	38.6	40.9	40.9
2	40.9	40.9	46.6	50
3	47.7	56.8	68.1	72.7
6	47.7	50	50	50
10	47.7	45.4	47.7	47.7
13	31.8	36.3	40.9	40.9
15	34.1	40.9	37.5	37.5
17	40.9	45.4	62.5	65.9
18	31.8	34.1	48.8	50
19	45.4	50	57.9	56.8

Subject	BIAfat1	BIAfat2
1	27	35
5	34	40
7	28	33
9	35	35
12	35	31
14	34	41
16	29	30
2	28	30
3	31	32
6	28	28
10	26	30
13	23	23
15	25	29
17	39	37
18	29	35
19	31	31

Subject	Bw 1	Bw 2
1	52	46
5	48	45
7	54	50
9	48	48
12	54	51
14	48	43
16	52	52
2	53	52
3	50	50
6	53	53
10	55	53
13	55	55
15	54	52
17	46	46
18	52	48
19	51	51

Subject	Vegetarian	Meat	Lax Exp	Strength Exp
1	Y		5	5
5	Y		3	24
7	N	Y	2	22
9	N		5	5
12	Y		4	24
14	N		3	5
16	N	Y	5	5
2	Y		5	24
3	N		2	5
6	Y		3	5
10	Y		6	24
13	N	Y	3	5
15	Y		4	5
17	Y		3	36
18	N	Y	3	5
19	Y		3	5

VITA

Megan L. Brenner was born in Vancouver, Canada where she was raised and educated. She graduated from Crofton House School for Girls in 1990. Megan continued her education at Whittier College in California where she recieved her Bachelor of Arts degree, majoring in Chemistry and French. While in college, she conducted research at the Treatment for Cancer and Blood Diseases Center, and was published in *The American Journal of Hematology* in July,1993, as co-author of 'Measurement of Red Blood Cell B12: A Study of the Correlation Between Intracellular B12 Content and Concentrations of Holotranscobalamin II'. She was also selected to the 1993 and 1994 Southern California All-Star Lacrosse Teams as well as the 1994 All-American Lacrosse Team and 1997 Canadian World Cup Lacrosse Squad.

Megan is currently completing her Master of Science Degree at Virginia Tech.