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The Effect of Creatine Monohydrate Supplementation on Anaerobic Performance During Sprint Training

by

Michael P. Gilpin 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Physical Education and Recreation

Edmonton, Alberta

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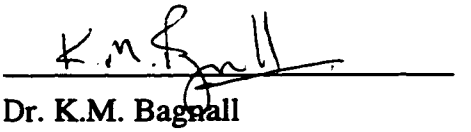

Dr. K.M. Bagnall

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Abstract

This study measured anaerobic performance at selected intervals during six weeks of sprint training on a cycle ergometer, and compared the effects of creatine monohydrate supplementation (C: n=9) with placebo values (P: n=10). Subjects performed variable numbers of sets of 10 s maximal sprints during three sessions each week, against a resistance of 100 g/kg of body mass. Results showed that training significantly ($p < 0.05$) increased peak, mean 5 s and 10 s power and repeated sprint performance equally in both groups. In addition, 5 min post-exercise blood lactate concentration values were the same for both groups. Throughout the study body mass was increased significantly ($p < 0.05$) in the C group. Supplementation resulted in significant increases in body mass but did not effect sprint performance and did not appear to alter the contribution of energy derived from glycolytic metabolism during maximal anaerobic exercise.

Dedication

To my parents for their help and support.

To Karen for making a pile of capsules.

To Cheryl for her love and support.

The journey was well worth the effort.

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Chapter One

Introduction

The ability to develop power during athletic performance is dependent on the rate of rephosphorylation of adenosine triphosphate (ATP), a vital component of repeated muscle contraction. However, rephosphorylation is limited by the availability of phosphocreatine (PCr) stores. The depletion of PCr during exercise results in a decrease in the ability to resynthesize ATP and a subsequent decrease in performance (Greenhaff, 1995). It has been suggested that if increased levels of PCr could be developed then ATP resynthesis and subsequent activity levels may be enhanced.

On the basis of this principle and information from both the common press and some scientific literature, some athletes use creatine monohydrate (CrH_20) in attempts to improve their physical performance. The process has been compared to the more common technique of carbohydrate loading which has proven to be very beneficial in endurance sports (McArdle et al., 1991). The goal of both processes is to increase the amount of substrate present in the muscle to levels above normal and then to utilize these high levels to the best advantage. It is suggested that individuals participating in sports which require demonstrations of single or repeated bouts of maximal force generation might utilize CrH_2O supplementation to increase artificially muscle total creatine stores (TCr = PCr + free Cr). Theoretically, it is possible that athletes may benefit from CrH_2O supplementation if the artificially increased TCr stores enhance the processes of energy release and resynthesis.

There appears to be few problems associated with CrH_2O supplementation which is fortunate considering its common use. Usually the only effect reported is an increase in body mass, but it remains unclear whether this increase can be attributed to an increase in lean tissue or water retention (Greenhaff et al., 1994; Balsom et al., 1995; Earnest et al.,

1995; Green et al., 1996; Mujika et al., 1996; Vandenberghe et al., 1997; Volek et al., 1997). However, the majority of Cr supplementation studies have been acute experiments, not exceeding seven days in duration, typically consisting of a pre-test, five day supplement loading phase, and a post-test (Balsom et al., 1993; Greenhaff et al., 1993; Harris et al., 1993; Birch et al., 1994; Greenhaff et al., 1994; Balsom et al., 1995; Cooke et al., 1995; Earnest et al., 1995; Casey et al., 1996; Odland et al., 1997; Kreider et al., 1998). Acute studies have concentrated primarily on the effects of Cr supplementation on physiological Cr concentrations and anaerobic exercise performance. No long-term studies appear to have been performed except for those completed by Sipila et al. (1981) and Vandenberghe et al. (1997). Sipila et al. (1981) reported the chronic use of low dose Cr supplementation in patients with the special problem of gyrate atrophy. Chronic low dose Cr supplementation inhibited the muscle atrophy associated with the affliction and no deleterious effects were reported. Vandenberghe et al. (1997) reported that 10 weeks of Cr supplementation in combination with resistance training produced superior strength increases compared to training alone. There is clearly a need to complete more long-term studies on the effects of creatine supplementation particularly in regard to performance during anaerobic exercise because of its current popular use by participants in such activities and the lack of supportive evidence that such supplementation is effective and safe.

Statement of the Problem

The use of creatine monohydrate (CrH_2O) as an ergogenic aid has been shown to increase anaerobic performance following acute short-term supplementation (Harris et al., 1993; Greenhaff, et al. 1993; Greenhaff, et al., 1994; Birch et al., 1994: Balsom, et al., 1995; Earnest et al., 1995; Casey et al., 1996). The use of CrH_2O supplementation during long-term training has only been investigated by Vandenberghe (1997) even though anecdotal reports, obtained from the popular press, indicate that CrH_2O supplementation is

being widely used by athletes engaged in sports requiring single or repeated movements involving maximal muscle force generation such as sprinters, weightlifters, throwers, football and hockey players. With such apparent widespread use, the effects and possible benefits of long-term CrH_2O supplementation clearly need to be explored since positive effects have frequently been reported with short-term use (Harris et al., 1993; Greenhaff, et al. 1993; Birch et al., 1994; Greenhaff, et al., 1994; Balsom, et al., 1995; Earnest et al., 1995; Casey et al., 1996). Therefore, the questions to be asked are: whether long-term CrH_2O supplementation can also enhance the training response; and, whether or not there are any harmful side-effects. Theoretically, it can be argued that CrH_2O supplementation would enhance the length of time that maximal force generation could be maintained by affecting the rate of ATP resynthesis (Greenhaff et al., 1993). Consequently, an individual participating in an event that requires rapid maximal energy expenditure or maximal efforts repeated in quick succession may benefit from CrH_2O supplementation.

Purpose

This study was designed to address three questions:

(1) Does CrH_2O supplementation during training affect the ability to generate peak and average muscular power during 10 s sprints?

(2) Does CrH_2O supplementation during training in combination with training affect repeated power output during maximal short-term exercise?

(3) Does CrH_2O supplementation during training affect post-exercise blood lactate concentration?

Null Hypothesis

Creatine monohydrate (CrH_2O) supplementation during six weeks of training will:

(1) Not have a positive effect on the ability to generate peak or average muscular power during 10 s sprints.

(2) Not improve performance during repeated 10 s sprints with limited recovery between sprints.

(3) Will not affect post-exercise blood lactate concentration in comparison to a non-supplemented group participating in an identical training program.

Limitations

(1) Failure to adhere to the CrH_2O supplementation schedule especially during the loading phase would adversely affect the quantity of Cr absorbed by the muscle.

(2) Failure to complete the training program would result in a reduced training stimulus.

(3) Accurate collection of urine samples is dependent on subject compliance.

(4) Application of resistance to the flywheel of the training and testing apparatus is manually applied through the use of a lever arm. Therefore, accurate application of the resistance is dependent on the skill of the investigator.

(5) Accuracy of measurement, e.g.; body mass, power output (W), and pipetting.

(6) Effectiveness of the assay procedure for determination of supplementation on creatine metabolism.

Delimitations

(1) All training and testing was supervised at the Exercise Physiology Laboratory at the University of Alberta.

(2) Subjects were encouraged to conduct all training sessions at the same time of day to minimize the possible effects of circadian rhythms on training.

(3) Subjects were required to have a history of participating in activities which require intense anaerobic exercise.

(4) Participants could not be involved in additional training or competition during the course of the study.

(5) All instruments were calibrated prior to each test and training session.

(6) Standards were analyzed to determine the accuracy of the assay and all samples were analyzed in duplicate.

Chapter Two

Literature Review

Historical Background

Creatine was identified and named by Chevreul in 1832, and was confirmed as a normal component of mammalian skeletal muscle by Leiberg in 1847. With the observation that creatine was related to muscle mass Lieberg suggested that creatinine found in urine was degraded creatine. The discovery that creatinine excretion was related to muscle mass led to the conclusion that the amount of creatinine in urine was a direct reflection of the quantity of creatine stored in muscle tissue, which suggested that retention of creatine by the body was possible. With the observation that not all of the creatine ingested was recovered in urine Folin and Denis (1912, 1914) concluded that creatine supplementation can increase skeletal muscle creatine concentration. Studies involving the electrical stimulation of cat muscle resulted in the discovery of phosphocreatine by Fiske and Subbarrow (1927). It was found that during electrical stimulation of muscle, phosphocreatine stores decreased and returned to resting levels during the subsequent recovery period. It is from this initial work that creatine and phosphocreatine were identified as key intermediates in skeletal muscle metabolism (Balsom, 1994).

"Immediate" Energy Metabolism

The concentration of ATP in muscle tissue is 8 mM (Houston, 1995). ATP concentrations can be easily maintained through a gradual acceleration of ATP-producing reactions such as fuel oxidation during slow transitions from resting to submaximal work rates. However, in muscle the maintenance of ATP homeostasis is problematic during intense exercise such as maximal sprinting since ATP hydrolysis may occur at a rate up to 4 mM per kg of muscle per second which would exhaust ATP stores within 2 s (Houston, 1995).

Phosphocreatine (PCr) is the molecule responsible for the regeneration of ATP during periods of rapid utilization. PCr transfers its phosphate group to ADP, and in the process produces ATP and creatine (Cr). This reaction, shown in Figure 1, is catalyzed by the enzyme creatine kinase (CK) (Houston, 1995).

$$ADP + PCr \xrightarrow{CK} ATP + Cr$$

Figure 1: Creatine kinase reaction

The creatine kinase reaction is freely reversible. The forward reaction is responsible for the regeneration of ATP during muscle contraction, while the reverse direction allows for the regeneration of PCr during recovery (Houston, 1995). Since the concentration of PCr in muscle is approximately 4 to 5 times that of ATP the creatine kinase reaction through the utilization of PCr acts as a temporary, or temporal energy buffer until alternate ATP regenerating processes, such as anaerobic glycolysis and oxidative metabolism are able to achieve maximal rates (Houston, 1995).

The phosphagen system also serves as a spatial energy buffer in muscle linking sites of ATP utilization with sites of ATP production. This mechanism has been called the phosphocreatine shuttle since it allows for the diffusion of Cr and PCr between mitochondrial sites of ATP production and utilization (Bessman et al., 1981). The phosphocreatine shuttle allows for the utilization of chemical energy, as well as its replenishment during exercise of skeletal muscle (Bessman et al., 1981).

It is possible that Cr functions to modulate the increase in glycolysis during intense exercise. During the onset of intense muscular work PCr is decreased due to the rapid need for replenishing ATP. Storey et al (1974) suggested that glycolysis may be stimulated by the decline in PCr levels. Phosphofructokinase (PFK) is the rate limiting enzyme for glycolytic reactions; however the action of PFK appears to be partially inhibited by the concentration of PCr. PCr concentration is decreased during intense exercise, which

stimulates PFK activity resulting in increased ATP production from glycolysis (Volek et al., 1996).

Biosynthesis of Creatine

Creatine is a nitrogenous organic compound attained through either the ingestion of exogenous sources, or endogenous synthesis. Exogenous sources of creatine can be attained from the consumption of animal protein (Volek et al., 1996). Endogenous creatine synthesis occurs primarily in the liver, pancreas, and the kidneys (Heymsfield et al., 1983) Skeletal muscle contains approximately 98 % of the total Cr pool of which 40 % is in the form free Cr with the remaining 60% in the form of PCr (Volek et al., 1996).

Cr is derived from three precursor amino-acids; arginine, glycine, and methionine. The synthesis of creatine begins with the formation of guanidoacetate from the amino-acids arginine and glycine (Heymsfield et al., 1983). The formation of guanidoacetate is controlled by the rate limiting enzyme glycine amidinotransferase (transmidinase), and the rate of Cr synthesis is regulated by the feedback inhibition of transamidinase. Consumption of a Cr-free diet causes this pathway to become fully activated. In this situation sufficient guanidoacetate is formed from the amino-acid precursors (Heymsfield et al., 1983). Transamidinase activity is modulated by testosterone which stimulates the *de novo* synthesis of transamidinase which increases the rate of production of quanidoacetate and Cr (Heymsfield et al., 1983).

In the subsequent reaction, Cr is formed by the transfer of a methyl group from S-adenosyl-methionine to quanidoacetate (Heymsfield et al., 1983). In contrast to the previous reaction, the formation of Cr is irreversible, not rate-limited and formation occurs primarily in the liver (Heymsfield et al., 1983). Following synthesis, Cr is released into the circulation where it is actively taken up by muscle and other tissues against a 200:1 concentration gradient (Heymsfield et al., 1983; Harris et al., 1992). The absorption of Cr

by skeletal muscle does not occur through simple diffusion (Fitch et al., 1966). The process of Cr absorption occurs through an active transport mechanism which is dependent on extracellular Na^+ and metabolic energy (Loike et al., 1986; Guimbal et al., 1993). The active uptake of synthesized Cr replaces 2.0 % of muscle TCr concentration each day (Heymsfield et al., 1983). In the 70 kg adult reference male 98 % of the body Cr stores are contained in skeletal muscle of which 1.5 to 2.0 % of the TCr concentration is replaced daily (Heymsfield et al., 1983). Following the absorption of Cr by skeletal muscle, Cr is converted to PCr and eventually degraded to creatinine. As creatinine is filtered by the kidneys it is then excreted in the urine (Volek et al., 1996). In addition, the rate of Cr uptake by muscle tissue is apparently enhanced in the presence of insulin (Haugland et al., 1975). In addition, Harris et al. (1992) noted that Cr uptake also appears to be enhanced in response to exercise.

Effect of Supplementation on Creatine Biosynthesis

Muscle Cr concentration appears to be highest in individuals who consume a protein-rich diet obtained from animal sources, while vegetarians display the lowest basal levels (Delanghe et al., 1989). Delanghe et al. (1989) reported that Cr levels for vegetarians were, mean ($\pm$ SE), 25.1 (9.1) umol/L, and 40.8 (19.0) umol/L for individuals who regularly included animal sources of protein in their diet.

The addition of Cr to the diet represses arginine-glycine transamidinase concentration (Walker, 1960). In addition, the repression of the catalytic sites specific to arginine and guanidinoacetate are also affected. It was concluded that the physiological repression of Cr synthesis served primarily to conserve the essential dietary amino-acids, arginine, glycine, and methionine, for protein synthesis (Walker, 1960). The rate of endogenous Cr synthesis is regulated by the feedback inhibition of transamidinase pathway which is fully activated during a Cr free diet and inhibited in the presence of increasing dietary Cr levels. The addition of dietary Cr partially or totally represses transamidinase

activity liberating the precursor amino-acids for other reactions such as protein synthesis (Heymsfield et al., 1983). It appears that *de novo* synthesis of Cr reactivates when exogenous Cr sources are removed and intramuscular stores are reduced to pre supplementation values (Crim et al., 1975; Crim et al., 1976; Walker, 1960; & Heymsfield et al., 1983).

Effect of Creatine Supplementation on Muscle Total Creatine Concentration

Harris et al., (1992) examined the effects that creatine monohydrate (CrH_2O) supplementation on the uptake and regulation of the TCr concentration of the human body. The purpose of the study was to determine whether dietary supplementation of CrH_2O could be absorbed by muscle tissue , and if continued supplementation could increase muscular PCr concentration. Results indicated that repeated five g doses every two hours for eight hours resulted in mean plasma Cr concentrations of 795 (SD 104) umol/L, and resulted in significant increases in total muscle Cr concentration (Harris et al., 1992). Increases in muscle Cr concentration were greatest during the first two days of supplementation. Renal clearance of Cr was 40, 61, and 68 % of the administered dose over the first three days of supplementation. Approximately 20 % of the total Cr absorbed was converted to PCr, but no changes in muscle ATP content was noted (Harris et al., 1992). The mean muscle TCr content of increased from 126.8 (SD 11.7) mmol/kg dry matter to 148.6 (SD 5.0) mmol/kg dry matter. The addition of one hour of one-legged cycle exercise significantly ($p < 0.05$) increased mean ($\pm$ SE) TCr in the exercised leg from 118.1(3.0) to 148.5 (5.2) mmol/kg dry matter in the control leg and to 162.2 (12.5) mmol/kg dry matter in the exercised leg. PCr concentration increased from 81.9 (5.6) to 93.8 (4.0) mmol/kg dry matter in the control leg and to 103.1 (6.2) mmol/kg in the exercised leg (Harris et al., 1992).

Effect of Short Term Creatine Supplementation

The majority of short term Cr studies are five to seven days in length and have used a variety of modalities to determine the effect of Cr supplementation on exercise performance and Cr metabolism. Results from several studies indicate that short term CrH_2O supplementation can significantly increase body mass. Body mass increases typically range between 1.0 to 2.0 kg ($p < 0.05$) following high dose supplementation of 20 to 30 g/d (Greenhaff et al., 1994; Balsom et al., 1995; Mujika et al., 1996; Cooke et al., 1997). The method of Cr supplementation previously described has been shown to be an effective way to rapidly increase muscle TCr concentration (Hultman et al., 1996). Several studies have demonstrated that short term supplementation can significantly elevate skeletal muscle TCr (Cr and PCr) concentration. Increases in TCr concentration range from 15 to 27 % following supplementation (Greenhaff et al., 1994; Balsom et al., 1995; Casey et al., 1996; Green et al., 1996; Rossiter et al., 1996; Odland et al., 1997).

The effect of CrH_2O supplementation on plasma ammonia and blood lactate concentrations have also been investigated. Both metabolites are indicators of high intensity exercise performance. Plasma ammonia concentration is considered a marker of adenine nucleotide loss while blood lactate concentration represents an indicator of the intensity of anaerobic exercise. The results from several studies are inconclusive regarding the effects of CrH_2O supplementation on plasma ammonia and blood lactate concentrations. Following short term supplementation several studies noted that post exercise plasma ammonia concentration was significantly ($p < 0.05$) decreased during maximal exercise (Greenhaff et al., 1993; Birch et al., 1994; Mujika et al., 1996). The decreased accumulation of plasma ammonia observed during exercise following supplementation supports the possibility of increased ATP resynthesis due to an increased availability of PCr which may allow for improved ATP maintenance during muscle contraction (Greenhaff et al., 1993; Greenhaff et al., 1993; and Greenhaff et al., 1994).

The effect of CrH_2O supplementation on post-exercise blood lactate concentration are contradictory. No change in post exercise blood lactate concentration was noted in several studies (Greenhaff et al., 1993; Birch et al., 1994; Greenhaff et al., 1994; Casey et al., 1996; Mujika et al., 1996; Odand et al., 1997). However, Balsom et al. (1995) noted a significant ($p < 0.05$) reduction in muscle lactate concentration determined from muscle biopsy samples. The reduction in post exercise muscle lactate concentration may indicate that CrH_2O supplementation delays the onset of ATP regeneration through glycolytic and oxidative processes.

The effect of short term CrH_2O supplementation on exercise performance has been examined using several modalities; however the ergogenic effects are not conclusive. Prior to and following CrH_2O supplementation Harris et al. (1993) noted that CrH_2O supplementation resulted in a reduction in 300 and 1000 m running time. Greenhaff et al. (1993) examined the effects of CrH_2O supplementation on skeletal muscle isokinetic torque during five bouts of maximal isokinetic exercise. Subjects who were supplemented with CrH_2O demonstrated greater muscle peak torque values for the final 10 contractions in exercise bout one ($p < 0.05$), for all contractions in bout two ($p < 0.01$), three ($p < 0.05$), four ($p < 0.057$), and during contractions 11 to 20 of the fifth exercise bout ($p < 0.05$). Results indicated that CrH_2O supplementation allowed for improved generation of muscle peak torque possibly resulting from an acceleration of PCr resynthesis. Additional work by Greenhaff et al. (1994) noted that CrH_2O supplementation significantly ($p < 0.05$) increased PCr resynthesis from 23 to 53 % following 120 s of recovery from intense electrically evoked isometric contractions of the vastus lateralis muscle.

Birch et al. (1994) investigated the effects of Cr supplementation during three 30 s bouts of maximal isokinetic cycling. Cr ingestion significantly increased peak power, mean power, and total work output during the first and second exercise bout. Cr supplementation did not affect performance during the third bout of exercise (Birch et al.,

1994). Balsom et al. (1995) examined the effect of high intensity exercise on a cycle ergometer. Subjects were evaluated for their ability to maintain peak power after a 40 s recovery interval following the completion of the 6 s exercise bouts. Subjects were also evaluated for performance in counter movement and squat jumps before and after supplementation (Balsom et al., 1995). Following the fifth exercise bout PCr concentration was higher 69.7 (2.3) mmol/kg dry matter for the Cr group compared to 45.6 (7.5) mmol/kg dry matter for the placebo group. The Cr supplemented subjects had superior performance compared to the placebo group in the maintenance of peak power during the 10 s exercise protocol. No change in counter movement or squat jump performance was noted for either the Cr or placebo group. Results indicate that Cr supplementation may improve resistance to fatigue during short term high intensity exercise. Balsom et al. (1995) concluded that the ability to maintain peak power may be associated with a greater availability of PCr and a decreased muscle lactate accumulation. However, the absence of an increase in jump performance may indicate that short-term Cr supplementation may not influence peak power generation. Volek et al. (1997) noted that following supplementation there were no differences in weight lifting, but significant increases were recorded for jump squat performance.

Febbraio et al. (1995) examined the effect of Cr supplementation on exercise metabolism and performance. Changes in intramuscular Cr concentration followed five days of Cr supplementation; in addition the effect of a 28 day wash out period was examined. Results indicate that following four, 1 min cycling bouts plus a fifth exercise bout to failure at 115 to 125 % of maximal aerobic power, no difference in exercise performance was noted following CrH_2O supplementation. Intramuscular TCr concentration was elevated following Cr supplementation; however there were no differences in the total adenine nucleotide pool, inosine 5'-monophosphate, ammonia, lactate, or glycogen contents compared to placebo ingestion. In addition, a 28 day washout period provided sufficient time to return intramuscular TCr stores to normal levels.

Febbraio et al. (1995) concluded that Cr supplementation may increase recovery from repeated bouts of high intensity exercise, but may not affect performance when aerobic metabolism is the principle energy supplier.

Cooke et al. (1995) examined the effect of Cr supplementation on power output and fatigue during cycle ergometry. The results from this study were in contrast to the findings of other research. Results indicate that no difference was observed for mean values of peak power, time to peak power, total work, and fatigue index following CrH_2O supplementation, during two, 15 s maximal sprints against a constant resistance with a rest interval of 20 min. It is possible that CrH_2O supplementation only conveys an ergogenic effect when the need for maximal energy production is interspersed with relatively short rest periods. However, further work by Cooke et al. (1997) indicated that CrH_2O supplementation had no effect during repeated maximal cycling performance regardless of rest interval length.

Mujika et al. (1996) examined the effect of CrH_2O supplementation on sprint performance in highly trained competitive swimmers. Subject were tested for sprint swimming performance on two different occasions separated by seven days. Testing consisted of three maximal swims of 25, 50, and 100 m from a competitive diving start. After the baseline session subjects were matched for performance level and assigned to either the CrH_2O (n=10) or placebo (n=10) group. No significant difference was noted for performance in any of the three swim trials or in the sum of the three trials in either group. The results Mujika et al. (1996) suggest that CrH_2O supplementation does not improve sprint performance in highly trained competitive swimmers. In addition, Rossiter et al. (1996) noted no difference in 1000 m rowing performance following five days of CrH_2O supplementation.

Casey et al. (1996) investigated the effects of Cr supplementation on skeletal muscle energy metabolism and performance during two bouts of maximal isokinetic cycling

exercise performed at 80 rpm and separated by four min of passive recovery. Results indicate that CrH_2O supplementation was not effective in improving isokinetic cycling performance. Odland et al. (1997) examined the effect of Cr supplementation on performance during a single 30 s bout of maximal cycling exercise. If Cr supplementation could increase skeletal muscle PCr content than it was hypothesized that this would increase the duration of peak and average power output. Exercise consisted of a one, 30 s Wingate test on a Monarch cycle ergometer. Resistance was set at 0.075 kg/kg of body weight. Power output was computed for each second of exercise, peak power (W/kg), mean 10 s power (W/kg), mean 30 s power(W/kg) and percent fatigue. Results indicate that no difference was observed between conditions for any of the exercise measures.

Effect of Long Term Creatine Supplementation

There are relatively few long term CrH_2O supplementation studies available (Sipila et al., 1981; Earnest et al., 1995; Vandenberghe et al., 1997; Kreider et al., 1998). Long term CrH_2O supplementation has shown similar results in comparison to the short term studies. Significant ($p < 0.05$) increases in body mass are similar to those demonstrated in short term studies (Sipila et al., 1981; Earnest et al., 1995; Vandenberghe et al., 1997; Kreider et al., 1998). Elevation ($p < 0.05$) of muscle TCr concentration, PCr/ATP ratio, and PCr concentration was noted following five and 10 weeks of CrH_2O supplementation (Vandenberghe et al., 1997).

Low dose CrH_2O supplementation for one year was found to reverse the effects of gyrate atrophy, which is characterized by progressive constriction of visual fields depression of creatine metabolism and type to muscle fiber atrophy (Sipila et al., 1981). Results indicated that chronic CrH_2O supplementation of 1.5 g/d for one year resulted in a 46 % increase in type two muscle fiber diameter (Sipila et al., 1981). Long term CrH_2O supplementation and exercise performance was investigated using a variety of test modalities. Earnest et al. (1995) examined the effects CrH_2O supplementation had on

muscular power and strength in weight trained males. Results indicated following 14 days of supplementation subjects supplemented with CrH_2O were able to achieve and maintain greater levels of anaerobic power during three 30 s Wingates. Maximal strength in the bench press increased by six % and total lifting volume increased by 26 % in the CrH_2O group following 28 days of supplementation. No significant differences were observed in performance measures for subjects in the placebo group (Earnest et al., 1995). Vandenberghe et al. (1997) noted that CrH_2O supplementation in conjunction with 10 weeks of strength training significantly ($p < 0.05$) increased exercise performance in untrained females. Results indicate that 1 RM strength was significantly ($p < 0.05$) increased in comparison to a placebo group for five of seven exercises. Isokinetic arm flexion torque was also significantly ($p < 0.05$) increased in comparison to the placebo group following five and 10 weeks of resistance training three times per week.

Effect of Creatine Supplementation on Aerobic Performance

Energy for prolonged continuous exercise is provided through aerobic energy metabolism. However, contributions from anaerobic metabolism are necessary during the onset of exercise, high intensity exercise, and during continually changing exercise intensity (Balsom et al., 1993). The purpose of this study was to investigate the influence of Cr supplementation on supramaximal treadmill runs to exhaustion, and a six km outdoor run over varying terrain. Following the habituation procedure subjects performed on two consecutive days a treadmill run to exhaustion at 120 % of VO_2 max, and a six km undulating outdoor terrain run. Results indicate that Cr supplementation did not enhance performance or increase maximal oxygen uptake during prolonged continuous exercise (Balsom et al., 1993).

Creatine supplementation has been shown to improve performance in short term maximal exercise although no performance improvements were noted when aerobic

metabolism was the dominant energy source. Stroud et al. (1994) suggested that Cr supplementation may influence the pattern of substrate utilization during prolonged submaximal exercise. Treadmill running was performed at 10 km/h at predetermined 6 min interval workloads from 50 to 90 % of VO_2 max. Results indicate that Cr supplementation had no significant effect on oxygen consumption, blood lactate concentration, or respiratory exchange ratio (Stroud et al., 1994).

Additional Information Regarding Creatine Supplementation

With the exception of Harris et al. (1992) little work has focused on dosage research regarding Cr supplementation. Hultman et al. (1996) examined the effects of different Cr dosages on skeletal muscle Cr accumulation. Hultman et al. (1996) sought to;
(1) characterize the increase and decrease of muscle Cr using previously established Cr loading protocols,
(2) determine if elevated Cr muscle Cr levels could be maintained by supplementing with a Cr dosage known to approximate muscle Cr degradation to creatinine,
(3) determine the effectiveness of low dose supplementation on elevating muscle Cr concentration,
(4) characterize the effects of Cr supplementation on urinary creatinine output.

Results indicate that no change in ATP concentration occurred. Significant increases in muscle TCr was composed mainly of free Cr however, increases in PCr were not significant. The rate of muscle TCr declined toward pre-supplementation concentrations over the 28 days after CrH_2O supplementation was halted. Following brief high dose supplementation low doses of 2 g per day were found to be sufficient to maintain elevated muscle TCr concentrations, and Cr ingestion of 3 g/d will in the long-term successfully raise muscle TCr to the same extent as short-term 20 g/d doses. Urinary creatinine excretion was documented during and after placebo and Cr supplementation. During the final 28 days of the study a large amount of variation was observed among

subjects when urinary creatinine output was compared. The average creatinine excretion was 2.8 mmol/day greater following Cr supplementation when compared to placebo trials. In addition, urinary volume output was reduced during the initial days of Cr supplementation.

The results from Hultman et al. (1996) indicate that the most rapid method to increase muscle Cr stores is through the utilization of a dosage equivalent to 0.3 g/kg body mass for six days, and maintained with a lower dose of 0.03 g/kg body mass. Results suggest that Cr entry into skeletal muscle is initially dependent on the extracellular Cr concentration but is subsequently down regulated in the presence of elevated extracellular and intracellular Cr concentrations. In addition, analysis of urinary creatinine excretion suggests that the rate of creatinine formation is directly proportional to the muscle Cr concentration indicating that the endogenous production of Cr may not be inhibited after Cr supplementation.

Vandenberghe (1996) suggested that intramuscular Cr and PCr could be further elevated through the addition of a direct stimulus to the membrane Cr transport system during Cr supplementation. Vandenberghe et al. (1996) compared the effects Cr supplementation with Cr supplementation in combination with caffeine (Cr+C). Results indicated that muscle ATP concentrations remained constant over the three supplement conditions. Cr and Cr+C increased ($p < 0.05$) muscle PCr concentration by 4 to 6 %. Muscle torque production was increased by 10 to 23 % ($p < 0.05$) by Cr supplementation. However, Cr+C supplementation failed to enhance muscle torque production. Therefore, the ergogenic effect of Cr supplementation appeared to be eliminated by caffeine consumption.

Further research by Greenhaff et al. (1996) and Green et al. (1996) investigated the effect of carbohydrate (CHO) consumption in combination with Cr supplementation on muscle Cr accumulation. Supplementation resulted in an increase in PCr and Cr in both

groups; however the increase in TCr was 60 % greater for the group which supplemented with CHO in addition to Cr. In addition, there was a corresponding decrease in urinary Cr excretion for the CHO+Cr supplemented group. Cr supplementation had no effect on serum insulin production; however CHO+Cr supplementation substantially elevated insulin concentration ($p < 0.001$). Therefore, Cr supplementation in combination with carbohydrates appears to augment muscle Cr accumulation.

Effect of Anaerobic Power Training on Physical Performance

Short duration high intensity activities such as sprinting, weightlifting, hockey, football, and field events are dependent on anaerobic energy metabolism. The ability to produce maximal energy expenditure is dependent on the immediate mechanism of ATP replenishment referred to as the ATP-PCr system (Bouchard et al., 1991). Although non-oxidative glycolytic and oxidative pathways also contribute to ATP replenishment, it is the ATP-PCr system which is the dominant factor for maximal energy production.

Bell et al. (1988) examined the effect of one-legged sprint training on intramuscular and non-bicarbonate buffering capacity. Nine subjects (n=8 males, n=1 female) completed 15 to 20 intervals of 20 s at 90 rpm, four days per week for seven weeks. A work to rest interval ratio of 1:3 was utilized. Resistance was set at 150 % of the initial power output which elicited one-legged VO_2max. Resistance was increased by 10 % when subjects could complete 20 intervals on two consecutive sessions.

Results indicate that intramuscular pH at rest and after four min of recovery from the anaerobic power test were not significantly altered with training. Intramuscular pH one min after exercise was significantly higher after training in both the trained and untrained leg. Blood lactate at rest and one min post-exercise was not significantly different with training. Following four min of recovery, blood lactate was significantly higher after exercise with the trained leg in comparison to the untrained leg. Non-bicarbonate buffering

capacity increased significantly from 49.9 (1.11) prior to training to 57.8 (1.99) umol HCL/ g/ pH after training for the trained leg. Non-bicarbonate buffering capacity was not significantly altered with training in the untrained leg. Peak five s power increased by 12.4 % and mean 60 s power increased by 14.5 % in the trained leg. Peak five s power increased by 5.4 % and mean 60 s power increased 7.4 % for the untrained leg. In addition, two legged VO_2 max increased significantly after training. Single leg VO_2 max for both the untrained and trained leg were increased significantly. However, the trained leg was significantly higher than the untrained leg.

Research emphasizing anaerobic training has noted that significant adaptations can occur. Medbo et al. (1990) observed that six weeks of treadmill training resulted in significant increases in anaerobic capacity. Twenty subjects were categorized into; untrained (n=6), endurance trained (n=6), and sprint trained (n=8) categories. Subjects were then divided into two groups; group A performed three, 2 min runs of continuous running. Group B performed eight, 20 s runs in each session at speeds which would elicit exhaustion in 35 to 40 s. The training sessions of group B were designed to stress high energy release rates.

No difference was noted for the maximal accumulated oxygen deficit between untrained and endurance trained individuals. However, the maximal accumulated oxygen deficit for trained sprinters was 30 % greater compared to non-sprint trained subjects. It appears that training which does not tax the anaerobic systems, such as endurance training, will not result in increased anaerobic performance (Medbo et al., 1990). Results of this study indicate that six weeks of high intensity anaerobic training was sufficient to increase anaerobic capacity by 10 %. The difference in anaerobic capacity between non-sprint trained and sprint trained individuals may be due to long term training in combination with a genetic predisposition for greater anaerobic performance (Medbo et al., 1990).

Linossier et al. (1993) investigated the effects of seven weeks of sprint cycle training on maximal power output. Previous work has shown that short periods of high intensity work can increase peak power production during sprint exercise. Intense exercise lasting only a few seconds is primarily dependent on anaerobic processes for energy production. According to Linossier et al. (1993) sprint training has been shown to be ineffective for inducing changes in high energy phosphate stores (ATP and PCr) in resting muscle. However, faster subjects such as national caliber sprinters have demonstrated the ability to cause greater levels of PCr depletion during intense exercise (Linossier et al., 1993). Subjects (n=10) participated in seven weeks of sprint cycle training. Training consisted of two sets of 5 s sprints interspersed with 55 s of rest, with subject completing four training sessions per week . Training intensity was maintained at 80 % maximal peak force. Subjects were allowed 15 min rest periods between each sprint set. Training sessions during the first week consisted of eight sprints in each set, and increased by one sprint every week so that each set in the final training week consisted of 13 sprints. The force velocity relationship was every week prior to a training session in order to adjust the braking force to ensure that subjects were training at 80 % of maximum force (Linossier et al., 1993).

Results indicate that sprint training increased peak power production and peak velocity by 25 %, and 30 s total work output by 16 %. Prior to sprint training the velocity reached with no load was related to resting muscle PCr levels. Training induced changes in velocity were noted only when PCr stores were lowest which suggest that low PCr concentration may be a limiting factor in the ability to attain high velocity (Linossier et al., 1993).

Stathis et al. (1994) examined the effect of sprint training on concentrations of muscle adenine nucleotides and their degradation products before and after 30 s of maximal cycling. Eight subjects (n=6 males, n=2 females) completed seven weeks sprint cycle

training. Training consisted of up to 10 maximal 30 s sprints interspersed with three min of recovery between sprints. Results indicated that peak power increased by 16.8 %, mean power increased by 11.8 % over a 30 s sprint. The seven week sprint training program did not however increase VO_2 max. After training the end-exercise muscle ammonia and IMP contents were decreased ($p < 0.05$). Muscle lactate and Cr concentrations were unaffected by the training program. Sprint training significantly effected muscle metabolite concentration during exercise and recovery. Following training there was a 52 % fall in ATP. In addition the exercise induced accumulation of IMP and ammonia was reduced ($p < 0.05$) by 41 and 52 %. Inosine concentrations increased ($p < 0.05$) during the recovery period following training. Training resulted in an increase in ammonia concentration at two min of recovery; however these levels tended ($p < 0.06$) to be lower 20 min after training. There was a greater increase ($p < 0.05$) in lactate concentration after training. Lactate concentrations were higher after exercise and during 2 to 20 min of recovery. Training also resulted in decreased ($p < 0.05$) plasma hypoxanthine concentrations at 45 and 60 min of recovery. Results suggested that the balance between ATP hydrolysis and resynthesis was improved as a result of sprint training. Recovery data indicates that sprint training may reduce muscle purine loss (Stathis et al., 1994).

Adaptations To Anaerobic Training

Sprint training has been noted to affect the distribution of muscle fiber types in individuals engaged in sprint training. Linossier et al. (1993) noted that seven weeks of sprint cycle training resulted in a decrease in the percentage of type two b muscle fibers. Allemeier et al. (1994) noted that six weeks of sprint cycle training resulted in a significant decrease in myosin heavy chain (MHC) two b and an increase in MHC two a. Therefore the data suggests that sprint cycle training may result in conversions within the fast twitch fiber population.

Sprint training has been shown to produce no changes in muscle ATP or PCr levels following training (Linossier et al., 1993; Stathis et al., 1994). Lactate dehydrogenase (LDH) activity has been noted to be higher in sprint trained compared to sedentary individuals (Linossier et al., 1993). Linossier et al. (1993) reported that resting phosphofructokinase (PFK), and LDH activity increased by 19 and 20 % following sprint training while oxidative enzyme activity remained unaltered. Gaitanos et al. (1993) noted that plasma catecholamine (epinephrine and norepinephrine) levels increased 13 times following 10, 6 s sprints. However, evidence is limited regarding adaptive responses of catecholamines to anaerobic training (Gaitanos et al., 1993).

Stathis et al. (1994) examined the effect of seven weeks of sprint training on adenine nucleotide metabolism which is essential for its role as a transducer of chemical energy to mechanical work. The precise regulation of muscle adenine nucleotide metabolism is imperative for the performance of high intensity exercise. During maximal exercise the rate of ATP hydrolysis exceeds the rate of resynthesis, resulting in the increased production of ADP. Excess ADP is then catabolized in a series of reactions which result in the formation of inosine 5-monophosphate (IMP) and ammonia. The accumulation of muscle IMP and ammonia are closely related to the magnitude of ATP degradation. The IMP produced may be degraded to inosine and may be further catabolized to hypoxanthine. Both inosine and hypoxanthine can diffuse across the sarcolemma and may represent a loss of purine base from the muscle. Nucleosides and bases that are lost from muscle cells are replenished either through slow energy consuming de novo synthesis, or through the less energy expensive purine salvage pathway (Stathis et al., 1994). The purine salvage pathway provides a mechanism for converting hypoxanthine to IMP, which is then reaminated through the reactions of the purine nucleotide cycle resulting in the resynthesis of ATP. Stathis et al. (1994) concluded that seven weeks of sprint cycle training resulted in an improved balance between ATP hydrolysis and resynthesis during sprint exercise. Although the mechanism for this

adaptation is uncertain, training resulted in reduced concentrations of IMP and inosine during recovery from high intensity exercise. In addition, the rate of muscle inosine accumulation and plasma hypoxanthine concentrations during recovery were also reduced following training which suggests that sprint training may inhibit muscle purine loss.

The maintenance of ATP homeostasis is problematic during maximal exercise since the rate hydrolysis can exceed the rate of rephosphorylation. PCr functions as a buffer linking sites of ATP utilization with sites of production and may modulate glycolysis during intense exercise. Creatine is an organic compound attained through daily nutrition or through the biosynthesis from the precursor amino-acids arginine, glycine, and methionine. Supplementation with CrH_2O represses *de novo* synthesis but appears to reactivate once exogenous sources are removed and intramuscular TCr levels return to normal. Creatine supplementation has been shown to increase muscle TCr concentration but have no effect on ATP content. Short-term supplementation has been shown to rapidly increase TCr concentration and may decrease the accumulation of plasma ammonia following exercise. However, the effects of short-term supplementation on blood lactate concentration and exercise performance are contradictory. Furthermore, supplementation appears to have no effect on performance when anaerobic metabolism is not the dominant energy source. Long-term supplementation has been shown to inhibit or reverse the condition of with gyrate atrophy. Limited research is available regarding long-term supplementation and exercise performance but positive results have been shown with resistance training.

Chapter Three

Methodology

Subjects

Twenty-five volunteers were recruited from the student population at the University of Alberta. Inclusion criteria included:

(1) A history of intense physical exercise at least three times per week for the past six months. Consequently, subjects would be accustomed to intense physical work and be adequately prepared for the physical demands of this study.

(2) A willingness to discontinue their regular training program for the duration of the study to eliminate the effects of cross-over or interference that may arise from additional training periods.

(3) Could not have used CrH_2O supplements for a minimum of 30 days prior to commencement of the study to ensure that muscle total creatine (TCr) levels were not artificially elevated due to the 28 day excretion period for CrH_2O (Febbraio et al., 1995; Hultman et al., 1996).

(4) Adult males, due to the greater variability in creatinine excretion reported in females during the second half of the menstrual cycle (Heymsfield et al., 1983).

Experimental Design

The experiment was conducted using a double-blind, matched-pairs design with randomized assignment of subjects to either a control or treatment group. Ethics approval was obtained, and all subjects completed a written informed consent prior to the start of the study (see Appendix A). The CrH_2O supplement and placebo capsules were created by the primary investigator and distributed to the subjects by the second investigator. The use of two investigators ensured that a double-blind procedure was maintained throughout the study. Subjects allocated to the control group (P) received the placebo and subjects

allocated to the treatment group (C) received CrH_2O. Prior to commencement of the training and supplementation program subjects completed one orientation (day 1) and three familiarization trials on the cycle ergometer on days 3, 5, and 8. Each trial consisted of five 10 s maximal effort sprints separated by 50 s of unloaded cycling on a Monark cycle ergometer. Twenty subjects were matched for peak power on day 5 and randomly assigned to either (P) or (C) groups. Following the completion of the final familiarization trial on day 8, subjects completed their first training session and received the first supplement packages. The schedule for the experimental design can be seen in Figure 2.

Supplementation Protocol

Createam™ brand CrH_2O was supplied by The NUTRASENSE Company™ (Lenexa, Kansas 66215). The CrH_2O was contained in a 5:1 ratio of CrH_2O to sucrose and distributed in gel capsules. The placebo was composed of a 5:1 ratio of sucrose to skim milk powder blended in a food processor and distributed in gel capsules. The placebo and CrH_2O capsules were indistinguishable in all respects. Subjects were given the appropriate number of capsules every three days. From days 8 to 12, subjects completed the high dose loading phase based on the formula:

(0.30 g/kg of body mass)

which has been shown to maximize muscle TCr accumulation (Hultman et al., 1996). Following the loading phase subjects, were put on a daily maintenance dose:

(0.075 g/kg of body mass)

for the remainder of the study.

Subjects were instructed during the loading phase (day 8 to 12) to divide each daily dose into four equal portions. For example, applying the loading dose formula for an 80 kg subject indicates that this individual would require a total of 24 g of CrH_2O/day. The 24

g/day dose (approximately 27 capsules) was divided into four, 6 g portions. One of each of the four portions was to be consumed at breakfast, lunch, dinner and immediately before bed. Since the supplement was distributed in capsules, instructions were provided regarding how many capsules were to be consumed in each portion. Subjects were instructed to dilute 250 ml of regular concentration orange juice with an equal portion of water and this drink was to be taken with the supplement to provide a carbohydrate solution to augment CrH_2O absorption (Green et al., 1996a; Green et al., 1996b). During the maintenance period (day 13 to 50) each daily dose was to be taken at breakfast with 500 ml of similarly diluted juice. Subjects were allowed unrestricted consumption of non-caffeine containing fluid during the study. Subjects were instructed to eliminate caffeine (coffee, tea, soda pop, and aspirin) from their diet during the study since caffeine has been shown to inhibit creatine absorption (Vandenberghe et al., 1996). Subjects were specifically requested to eliminate coffee, tea, soda pop, and aspirin from their diet since these substances could easily be consumed in quantities similar (5 mg/kg body weight) to that used by Vandenberghe et al (1996). Chocolate was not specified as a restricted food due to its low caffeine concentration (Whitney et al., 1993).

Familiarization Procedure and Training Program

All training and testing was conducted at the Exercise Physiology Laboratory at the University of Alberta under the supervision of the investigator. Subjects reported to the laboratory on Monday (day 1), Wednesday (day 3), and Friday (day 5) during the familiarization week. Body mass was recorded (kg) using a balance beam scale which was zeroed prior to each test session. The body weight recorded on the first familiarization trial was used to determine the resistance setting on the cycle ergometer for the duration of the study. Resistance was set according to the formula:

body mass (kg) x 0.100 kg,

which has been shown to elicit maximal power output (Bar-Or, 1987; Evans et al., 1981; Gilpin et al., 1996; Smith, 1987). Subjects adjusted the modified Monark Cycle Ergometer for comfortable seat height and handle bar placement. Settings for seat height were recorded to ensure consistency during the study. A five minute cycling warm-up at self-selected resistance's was performed prior to each test and training session in addition to self-administered stretching exercises for the quadriceps, hamstrings, and calves.

Familiarization trials (days 3, 5, and 8) consisted of five, 10 s sprints at a resistance setting of 100 g/kg of body weight separated by 50 s of unloaded cycling. Resistance was manually applied to the cycle ergometer using a lever arm. The cycle ergometer was calibrated at the beginning of each test day. The "drop method" of resistance application was utilized in which the subjects received a 5 s countdown to maximize the flywheel revolutions before the resistance was rapidly applied. Subjects were then vigorously encouraged to maintain maximal effort pedaling for 10 s.

The training and supplementation program commenced two days after completion of the familiarization period (day 8). The training program consisted of three supervised sessions per week for six weeks. Each training session involved repeated sets, of four to six, 10 s maximal effort sprints. Each sprint was separated by 50 s of unloaded cycling. Upon completion of each set of sprints, subjects were given a five minute period of active recovery consisting of easy cycling and stretching. The volume of training was applied according to the principles of periodization (Bompa, 1992: see Appendix B). The total volume of training increased from 270 s of sprinting in week one to 700 s of sprinting in week five. Training volume was reduced to 570 s during the sixth week to facilitate recovery as recommended by Bompa (1992) prior to the final exercise test on day 50.

Anaerobic Testing

Anaerobic testing was performed on a Monark cycle ergometer modified for anaerobic work and took place on days 1, 3, and 5 of the familiarization week and on days 8, 22, 36, and 50 of the experimental period. The cycle ergometer was fitted with toe clips, a racing-saddle, and a modified seat post to improve vertical and horizontal adjustment. The flywheel was fitted with a rheostat which was connected to a computer allowing measurement of flywheel revolutions and computation of power (W) for each s of exercise. Performance data were analyzed for peak, 5 s, and 10 s power. Peak power was determined as the highest power output recorded during any second of a 10 s sprint. Five s power was calculated as the average performance recorded during the first five seconds of a 10 s sprint. Ten s power was determined as the average power recorded over a 10 s sprint.

Urine Collection and Analysis

Each subject was provided with a 4 litre container for the purpose of urine collection. Subjects were instructed to collect their urine in the container upon waking on days 7, 14, 21, 35, and 49, and finish collecting urine upon wakir g on days 8, 15, 22, 36, and 50. The collected urine was brought to the laboratory on days 8, 15, 22, 36, and 50. Collected urine was measured for volume and a 5 ml sample was withdrawn and stored at -20 ^{o}C for later analysis. Containers were sterilized and returned to the subject. The assay used in this experiment was an enzymatic method for the determination of creatine and creatinine concentration in urine (Wahlefeld et al., 1974). See Appendix C for a complete description of the assay procedure.

Blood Collection and Analysis

Blood collection was performed by a registered nurse on days 1, 3, 5, 8, 22, 36, and 50. Following completion of the fifth sprint of the test protocol, subjects remained on the cycle ergometer and completed exactly three minutes of unloaded cycling. Subjects then immediately dismounted the ergometer and were seated for blood sample collection at five minutes post-exercise by venous puncture of a forearm vein. Immediately following the withdrawal of the blood sample, 0.20 ml of blood was placed in a preservative tube (YSI2372 preservative tube, Yellow Springs Instruments) and vortexed to ensure thorough mixing of the blood sample and the preservative. Following this procedure samples were stored at -20 ^{o}C until analysis following day 50.

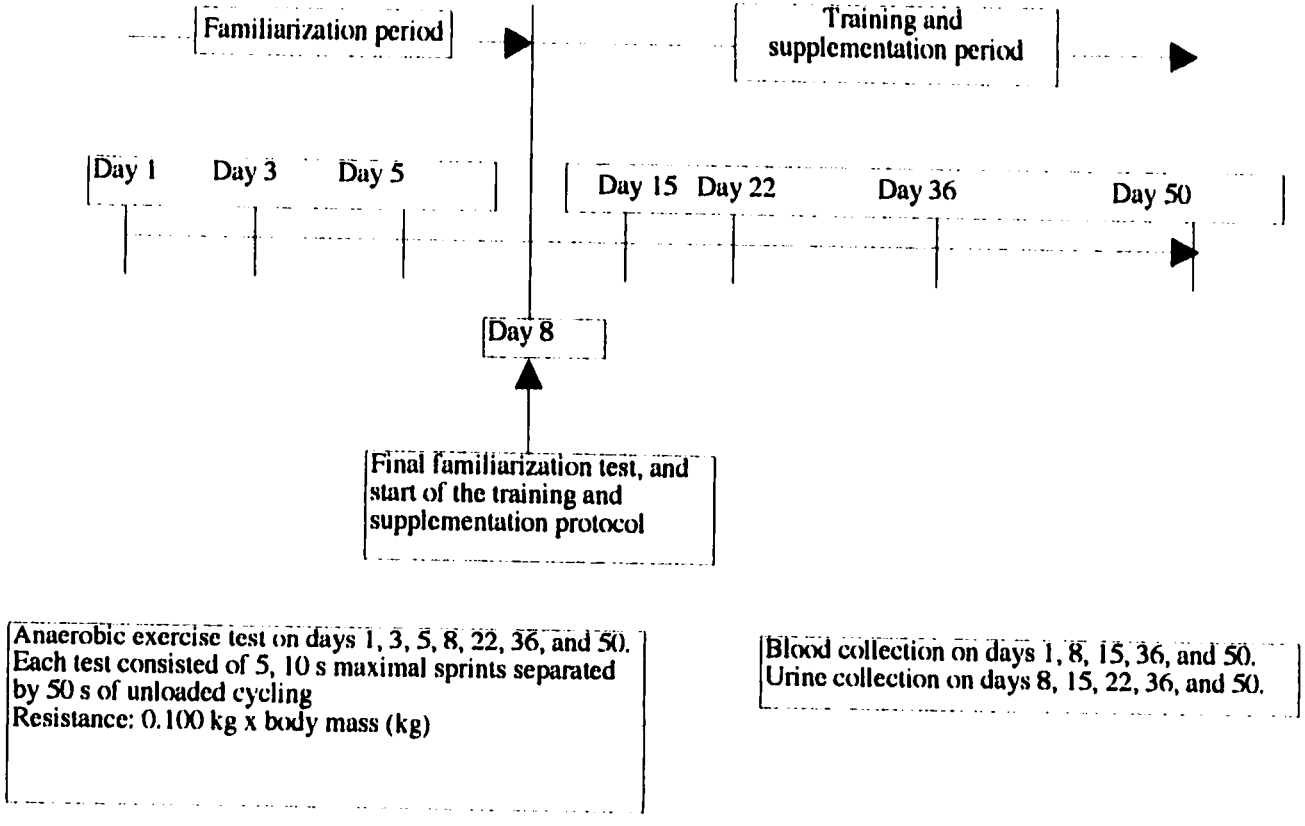
Blood samples were allowed to thaw at room temperature and were subsequently analyzed in duplicate using a Yellow Springs Instrument Model 27 lactate analyzer which was calibrated with lactate standards of known concentration. Samples were analyzed in sequential order with a validation calibration performed after every two samples. Analysis of duplicates proceeded in reverse order with calibration every two samples. This procedure ensured that each subject's samples were analyzed in the same batch and that a calibration occurred prior to analysis of each sample.

Statistical Analysis

Statistical analysis was conducted using Statview SE+ (Abacus Concepts) computer program. A two way ANOVA with repeated measurements with Scheffé post hoc analysis was used to determine if differences were present between groups over time. If no differences were observed between groups the data for both groups were collapsed into one group and a one way ANOVA with repeated measurements was conducted to determine if differences were observed across time. In addition, statistical analysis of data was conducted using both absolute values and change values. Change values were determined

as the difference from mean baseline values obtained prior to the commencement of the training and supplementation program. Where the results of the analysis of "absolute" and "change" data were the same, only the absolute values were presented for simplicity.

Figure 2: Experimental Schedule



Chapter Four

Results

Subject Attrition

Twenty-five subjects were initially recruited for the study. Following the orientation trial (day 1) and the second familiarization trial (day 3) five subjects withdrew for various reasons. Twenty subjects were matched for peak power and commenced the training and CrH_2O program on day 8. One subject was removed from the study for failing to comply with the CrH_2O loading protocol and training program. Nineteen subjects completed all phases of the study.

No major problems were encountered during this study. Minor problems with equipment occurred but they were easily fixed. The standard pedals on the Monark cycle ergometer were replaced with those of a more durable construction to withstand the power output generated by the subjects. The crank, handle bars, and tension strap on the Monark ergometer had to be replaced due to material fatigue. Blood and urine collection occurred without any difficulty.

Familiarization Performance

Subjects completed one orientation (day 1) and then three familiarization trials (days 3, 5, and 8) prior to commencement of the training and supplementation program on day 8. No differences were observed between groups for body mass during the familiarization period (Table 1). No differences were observed for peak power output when results from the second and third familiarization trial were compared (Table 1).

Body Mass

Following the training and supplementation program no difference was recorded in average body mass for the P group. In contrast, a significant increase of 2.0 (0.3) kg ($p <$

0.05) in body mass was noted for the C group between day 8 and day 50 during the experimental period. Body mass increases ranged from 0 to 4.4 % for the C group. The greatest change in body mass increase for the C group occurred during the first 14 days of the experimental protocol (Figure 3).

Peak Power Output

No difference in peak power output was noted between the P and C groups following the CrH_2O supplementation and training program (day 8 to 50). However, significant ($p < 0.05$) increases in peak power were noted for both the P and C groups over the training period (Table 2).

5 s Power Output

No significant differences in 5 s power were noted between P and C groups following CrH_2O supplementation in combination with sprint training (day 8 to 50). However, significant ($p < 0.05$) improvements in mean 5 s power output were noted for both groups following sprint training (Table 3).

10 s Power Output

No significant differences in 10 s power output was noted between P and C groups following CrH_2O supplementation in combination with sprint training (day 8 to 50). However, significant ($p < 0.05$) improvements in mean 10 s power output were noted for both P and C groups following sprint training (Table 4).

Blood Lactate Data

Lactate concentration was determined after testing on day 1, 8, 22, 36, and 50 of the experiment. Mean blood lactate concentration was not significantly different before and after training for both P and C groups (Table 5).

Urine Output and Analysis

Urine volume for P and C groups displayed different patterns pre and post supplementation. Urine samples were collected on days 8, 15, 22, 36, and 50. A decrease in average urine volume output of 24.3 % was noted following the CrH_2O loading phase (days 8 to 15) for the Cr group but this decrease was not statistically significant (Figure 4). Significant differences in urine output between groups were noted on day 22. Analysis of urine samples demonstrated that CrH_2O supplementation significantly ($p < 0.05$) increased creatine excretion for the C group by 178-257 % compared to the P group (Table 6). No difference in creatinine excretion was noted between P and C groups (Table 7).

Effect of Sprint Training on Performance

Since there were no statistical differences in performance between the P and C groups, the performances of all 19 subjects were considered as one group. Sprint training resulted in a significant increase ($p < 0.05$) in peak power output by 7, 9 and 11 % from day 8 to days 22, 36 and 50 (Table 8). Performance improvements were observed for repeated sprints for both 5 s and 10 s performance measurements. Five s power outputs significantly increased ($p < 0.05$) by 15 and 18 % for sprints four and five (Table 9). Ten s power outputs significantly increased ($p < 0.05$) by 12, 15, 21, and 25 % for sprints two to five (Table 10). Improvements in performance increased progressively from sprint one to five (Table 9 and 10).

Table 1. Body mass and 1 s power output (mean ± SE) for the Placebo (P, n=10) and Creatine (C, n=9) groups during the familiarization period (Day 3, 5, and 8).

Group	Day 3	Day 5	Day 8
Body Mass (P)	79.8 (4.2)	78.5 (4.2)	78.5 (4.2)
Body Mass (C)	79.8 (5.8)	79.5 (5.8)	80.0 (5.8)
Peak Power (P)	1255 (73)	1343 (101)	1343 (94)
Peak Power (C)	1353 (102)	1404 (86)	1384 (68)

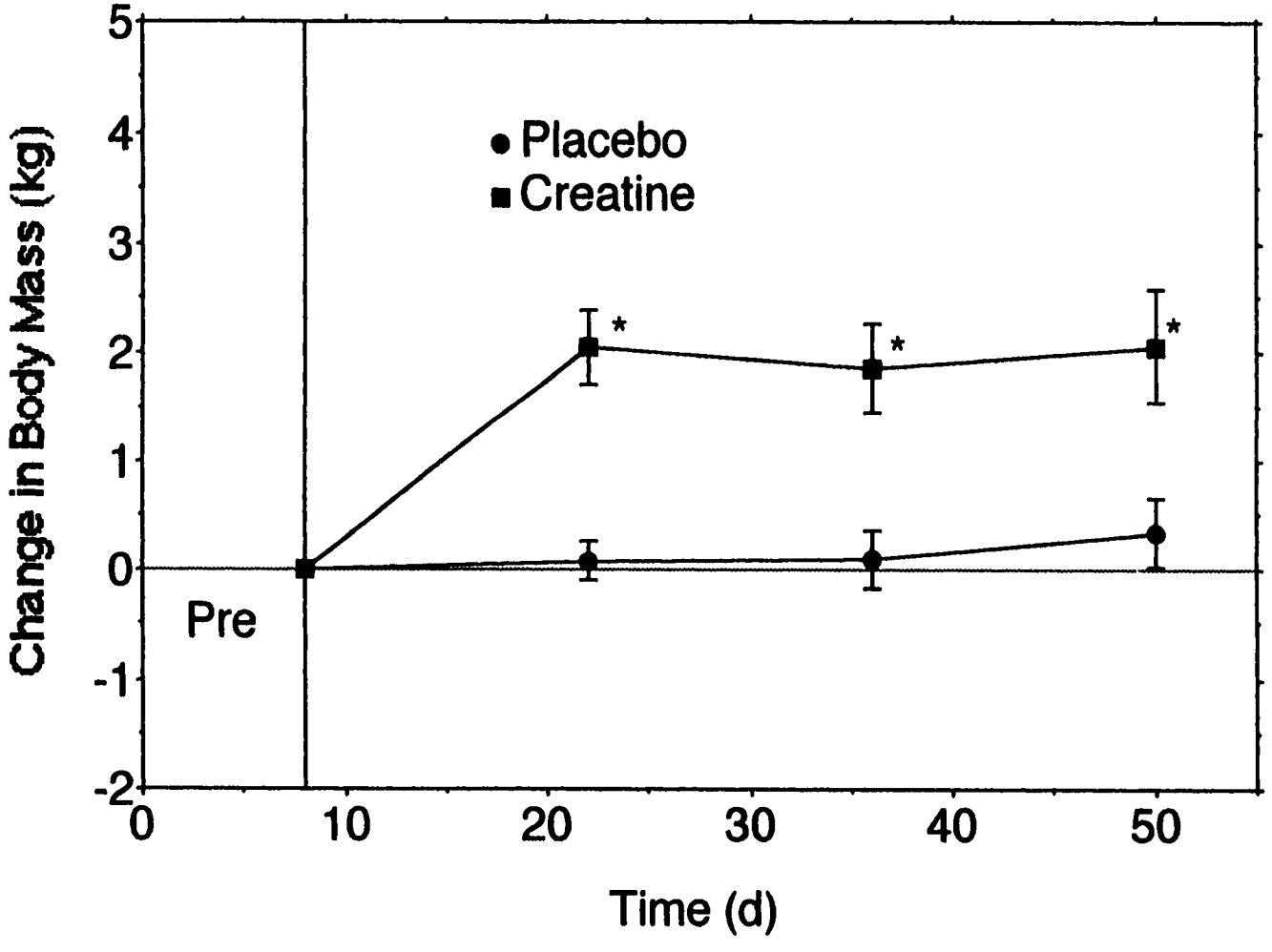


Figure 3. Change in Body Mass (mean kg ± SE) during pre to post training and CrH_2O supplementation. Pre indicates mean stable body mass before training and supplementation period for days 3, 5, and 8. Significant difference ($p < 0.05$) from pre and between groups denoted by *.

Table 2. Peak power output (mean ± SE) of Placebo (P) and Creatine (C) groups before (Pre: days 3, 5, and 8) and during the training and CrH_2O supplementation period (Days 22, 36, and 50).

Group	Pre	Day 22	Day 36	Day 50
P (n=10)	1314[a] (94)	1452 (90)	1434 (90)	1481[a] (88)
C (n=9)	1380[bc] (81)	1433 (69)	1508[b] (92)	1515[c] (84)

- Significant difference ($p < 0.05$) denoted by matching superscript letters.
- Pre represents the mean power output from days 3, 5, and 8.

Table 3. Average 5 s power output (mean ± SE) for Placebo (P) and Creatine (C) groups before (Pre: days 3, 5, and 8) and post (Day 50) training and supplementation.

Group	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
P Pre	1074 (65)	1030 (58)	953 (49)	909 (42)	863[a] (49)
P Post	1119 (48)	1134 (67)	1046 (53)	1020 (60)	990[a] (57)
C Pre	1012 (36)	1030 (41)	931[b] (36)	853[c] (53)	813[d] (55)
C Post	1098 (43)	1105 (49)	1048[b] (57)	1012[c] (51)	986[d] (55)

- Significant difference ($p < 0.05$) denoted by matching superscript letters.
- Pre represents the mean power outputs from days 3, 5, and 8.

Table 4. Average 10 s power output (W) (mean ± SE) for Placebo (P) and Creatine (C) groups before (Pre: days 3, 5, and 8) and post (Day 50) training and supplementation.

Group	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
P Pre	945^{a} (52)	845^{b} (45)	766^{c} (40)	714^{d} (33)	671^{e} (38)
P Post	1011^{a} (47)	958^{b} (54)	878^{c} (41)	851^{d} (48)	832^{e} (47)
C Pre	892^{f} (27)	831^{g} (29)	751^{h} (34)	677^{i} (42)	642^{j} (41)
C Post	999^{f} (38)	918^{g} (38)	862^{h} (44)	832^{i} (42)	808^{j} (41)

- Significant difference ($p < 0.05$) denoted by matching superscript letters.
- Pre represents the mean power outputs from days 3, 5, and 8.

Table 5. Blood lactate concentration (mmol/l ± SE) at 5 min post exercise for Placebo (P) and Creatine (C) group.

Group	Day 1	Day 8	Day 22	Day 36	Day 50
P (n=10)	12.9[a] (0.6)	13.0[b] (0.6)	11.4[abc] (0.8)	11.9 (0.7)	13.5[c] (0.8)
C (n=8)	13.8[e] (0.8)	13.7[f] (0.8)	11.8[efg] (0.9)	13.8 (0.6)	14.4[g] (0.4)

• Statistical difference ($p < 0.05$) denoted by matching superscript letters

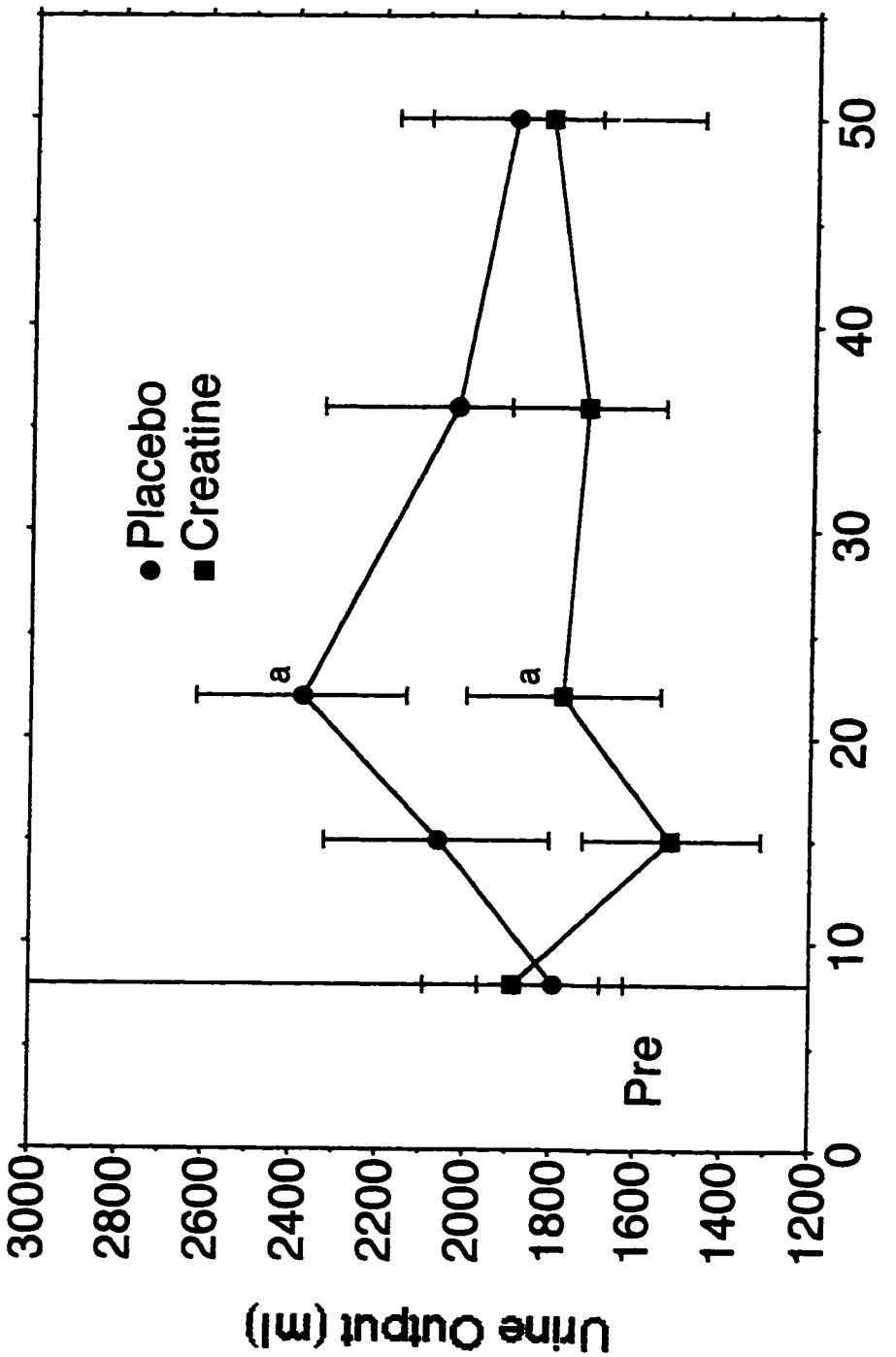


Figure 4. Urine output (mean ml ± SE) for Placebo (P) and Creatine (C) groups from pre (day 8) and during CrH_2O supplementation and sprint training (days 22, 36, and 50).

Table 6. Urinary creatine output (mean g/d ± SE) for Placebo (P) and Creatine (C) groups during the experimental phase. Day 8 values are prior to the start of CrH_2O supplementation. Day 15, 22, 36, and 50 represent the effect of CrH_2O supplementation.

Group	Day 8	Day 15	Day 22	Day 36	Day 50
P (n=8)	2.10 (0.32)	1.85^{a} (0.33)	2.36^{b} (0.34)	2.33^{c} (0.38)	1.72^{d} (0.26)
C (n=8)	3.04^{efgh} (0.70)	9.97^{ae} (2.87)	8.78^{bf} (1.74)	8.45^{cg} (1.65)	10.85^{dh} (3.26)

• Significant difference ($p < 0.05$) denoted by matching superscript letters

Table 7. Urinary creatinine output (mean g/d ± SE) for Placebo (P) and Creatine (C) groups during the experimental phase. Day 8 values are prior to the start of CrH_2O supplementation. Day 15, 22, 36, and 50 represent the effect of CrH_2O supplementation.

Group	Day 8	Day 15	Day 22	Day 36	Day 50
P (n=8)	2.38[a] (0.47)	2.32 (0.31)	3.65 (0.60)	2.50 (0.38)	2.43 (0.42)
C (n=8)	5.48[a] (1.17)	3.68 (1.62)	4.43 (1.00)	4.04 (1.04)	4.33 (1.16)

• Significant difference ($p < 0.05$) denoted by matching superscript letters

Table 8. Effect of sprint training on peak power output (mean W ± SE) for n=19 subjects before (Pre: days 3, 5, and 8) and during training (Days 22, 36, 50).

Pre	Day 22	Day 36	Day 50
1345^{abc} (58)	1443^{a} (57)	1469^{b} (63)	1497^{c} (59)

- Significant difference ($p < 0.05$) denoted by matching superscript letters.
- Pre represents the mean power output from days 3, 5, and 8.

Table 9. Effect of sprint training on 5 s power output on sprints 1 through 5 (mean ± SE) for n=19 subjects before (Pre: days 3, 5, and 8) and during training (Days 22, 36, 50).

Time	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
Pre	1044 (38)	1030 (35)	943 (30)	883[a] (33)	839[b] (36)
Day 22	1068 (36)	1054 (37)	1010 (37)	958 (32)	932 (33)
Day 36	1062 (39)	1022 (39)	1002 (39)	957 (35)	926 (36)
Day 50	1109 (32)	1120 (41)	1047 (37)	1016[a] (39)	988[b] (39)

- Statistical difference ($p < 0.05$) denoted by matching superscript letters.
- Pre represents the mean power output from days 3, 5, and 8.

Table 10. Effect of sprint training on 10 s power output on sprints 1 through 5 (mean W ± SE) for n=19 subjects before (Pre: days 3, 5, and 8) and during training (Days 22, 36, 50).

Time	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
Pre	920 (30)	838[a] (26)	759[c] (25)	697[d] (25)	658[efg] (27)
Day 22	943 (29)	865 (28)	815 (27)	768 (24)	743[e] (26)
Day 36	946 (31)	846b (32)	816 (31)	772 (28)	746[f] (28)
Day 50	1005 (30)	939[ab] (33)	871[c] (29)	841[d] (31)	821g (30)

- Statistical difference ($p < 0.05$) denoted by matching superscript letters.
- Pre represents the mean power output from days 3, 5, and 8.

Chapter Five

Discussion

The study of creatine monohydrate supplementation during a controlled training program was of interest because of the limited amount of longitudinal research on the use of this substance as an ergogenic aid. A training program consisting of repeated short-duration maximal effort sprints was chosen because this type of effort is characteristic of many sports and is theoretically very suitable for creatine supplementation. A strength of the program design was the focus on speed rather than resistance overload during the repeated sprint exercise. That is, increases in power output were accomplished by increased pedaling frequency against a fixed resistance throughout the six weeks of training and testing. A second important characteristic of the program was that the volume of training was to some degree determined by each subject. As noted, flywheel resistance was consistent for each subject, and all subjects completed exactly the same number of repetitions and sets with the same relief periods. However, by allowing the subjects to determine pedaling cadence, individual control of intensity and total work was possible. The mechanism(s) through which CrH_2O supplementation may enhance performance are not clearly defined and several possibilities exist. For example, if supplementation impacts the concentration of PCr and/or the rate of resynthesis, then logically, more work may be accomplished during a training session of repeated maximal effort work periods separated by limited rest periods. It is reasonable to presume that the training effect may be mediated by the quantity of work accomplished, which in turn is mediated by factors such as energy supply and recovery. In theory, if the C group was capable of more work during the training period, then it seems reasonable that the training effect may be manifest in enhanced power output during the sprint test.

The major finding of this study was that CrH_2O supplementation in combination with training failed to improve sprint performance when compared to the effects of training

alone. This outcome is in contrast with the results of the three other training studies currently available (Earnest et al., 1995; Vandenberghe et al., 1997; Kreider et al., 1998). These studies reported increases in maximal strength and muscular endurance during weight lifting exercise. In addition, Earnest et al. (1995) reported increases in performance during three consecutive 30 s Wingate tests and Kreider et al. (1998) reported improvements in performance during the first five of 12, 6 s sprints on a cycle ergometer. The results of the present study are in agreement with acute studies (Cooke et al. 1995; Mujika et al. 1996; Odland et al. 1997; and Cooke et al. 1997) which showed no improvements in performance with creatine supplementation.

Body Mass and Urine Output

The significant increase of 2.0 (0.3) kg in body mass in the C group noted during this study is similar to the increases reported (0.7 to 2.42 kg) in previous research with CrH_2O supplementation (Greenhaff et al., 1994; Balsom et al., 1995; Earnest et al., 1995; Green et al., 1996; Mujika et al., 1996; Vandenberghe et al., 1997, Volek et al., 1997). However, it remains to be determined if the body mass increases resulted from water retention, or increased mass of fat or muscle tissue. Kreider et al. (1998) using dual x-ray absorptiometry methodology (DEXA) determined that 28 days of training with CrH_2O supplementation resulted in significant increases in fat and bone-free mass compared to a placebo group. However, results from the present study suggest that the rapid increase in mass noted in the C group (Table 2) is associated with decreased urine output (Figure 2).

Present data cannot fully account for changes in fluid balance, however fluid retention in the C group presents a very plausible explanation for the mass changes for two reasons. First, muscle hypertrophy is generally believed to result after relatively long training periods of resistance training (Komi 1986). Therefore, it would be remarkable to attribute the mass increase ($p<0.05$) for the C group after only two weeks of sprint training (Figure 3) to hypertrophy. This is underscored by the observation of no significant mass

increase in the P group (training only) after six full weeks of training, and logically suggests that some other factor must account for the increased mass of the C group.

Secondly, the rapid increase in mass for the C group appears to parallel the decrease in urine output. All subjects ingested their daily dose of capsules with similar, specified quantities of dilute citrus juice. The data in Figure 3 show opposite patterns of change for urine output during the supplementation period. The urine volumes collected on Day 8 were intended to reflect the pre-supplementation baseline. When compared to Day 8, average urine output on Day 15 was decreased by 369 ml (20%) for the C group and increased by 270 ml (15%) for the P group. Considered simultaneously with the body mass results, these data suggest that the P subjects were excreting extra fluid and the C subjects were retaining extra fluid. The average decrease in urine output coincided with increase in body mass in the C group during the creatine loading phase, which suggests that the increased body mass was due in part to increased fluid retention (Table 2).

Interpretation of these data is limited by the lack of accurate daily fluid balance records. Strict monitoring of all fluid intake and excretion during the study would have been interesting, but probably an unreasonable expectation of the participants. However, based on the available data, if subjects in the C group retained an average of approximately 350 ml of fluid/day during the loading period, then the two kilogram increase in mass would be accounted for.

The observations regarding urine output in this experiment are consistent the results of Hultman et al. (1996). Anecdotal reports from users (Brunner, 1995) and advertisements from the sellers of creatine supplements frequently include testimonials for rapid increases in muscle mass. However, the available data strongly suggest that any short term change in body mass is probably better explained by altered fluid balance.

Peak Power Output

Although both groups increased peak power output, training in combination with CrH_2O supplementation did not result in a significant increase in performance compared to the results obtained through training alone. Studies utilizing short-duration sprint training programs, without CrH_2O supplementation, have reported increases in peak power output between 3 and 25% (Linossier et al., 1993; Allemeier et al., 1994; Stathis et al., 1994). In the current study, peak power increased on average, by 12.8% for the P group and 9.8 % for the C group. When considered individually, the range of improvement for the 19 subjects was from 3 to 57%.

Although modest increases in peak power (10 - 13%) were noted for both P and C groups due to training, CrH_2O supplementation did not provide an additional benefit compared to training alone. Data obtained in this study are in accordance with the results of acute studies reported by Balsom et al. (1995); Cooke et al. (1995); and Odland et al. (1997), which showed no significant increases in peak power generation following acute CrH_2O supplementation. However, CrH_2O supplementation has been shown to increase peak torque generation during isokinetic dynamometry, and maximal strength during weight lifting (Greenhaff et al., 1993; Earnest et al., 1995; Vandenberghe et al., 1997; Kreider et al., 1998). The changes in peak power observed with supplementation do not appear to be superior to that obtained by training alone (Sharp et al., 1986; Bell et al., 1988; Linossier et al., 1993; Linossier et al., 1997).

5 s Power Output

Sprint training resulted in a significant increase in 5 s power output for both groups. Power output changed by 4, 10, 10, 12, and 15% in the P group, and by 9, 7, 13, 19, and 21% for sprints one to five respectively, for the C group. Although there were no significant differences between P and C groups following training, only sprint five for

the P group demonstrated a significant increase from day 8 to 50 while the C group demonstrated a significant increase for sprints three to five. In contrast, Earnest et al. (1995) noted that power output during 5 s work intervals were significantly higher during all post-test trials during three consecutive 30 s Wingate tests separated by 5 min rest intervals following 14 days of supplementation and weight training. Kreider et al. (1998) noted a significant increase in the first five of 12, 6 s sprints separated by 30 s of recovery. Interestingly, the study by Kreider et al. (1998) combined supplementation with both sprint and weight training, Earnest et al. (1995) used weight training only, and the present experiment used sprint training only. Perhaps increasing muscular strength through weight training is an important determinant for increasing power output during cycling.

10 s Power Output

As with peak and 5 s data, training resulted in a significant increase in 10 s power output for both groups. However, no difference in performance was observed between groups due to the CrH_2O supplementation. Subjects in the P group improved by 7, 12, 15, 19, and 24% for sprints one to five respectively, while the C group improved by 12, 11, 15, 23, and 26% for sprints one to five respectively (all $p<0.05$). Improvements in performance demonstrated the same pattern as observed with the 5 s power output data. Therefore, this experiment indicated that CrH_2O supplementation did not provide an effective ergogenic aid for the improvement of repeated sprint cycle performance over the effects obtained by training alone.

Creatine and Creatinine Excretion

Creatine output remained unchanged for the P group over the course of the experimental protocol. Mean creatine excretion was significantly increased for the C group at days 15, 22, 36, and 50 (post-CrH_2O supplementation) when compared to day 8 (pre-CrH_2O supplementation). During this period, mean creatine excretion increased by an

average of 213% compared to day 8 values for the C group. Following completion of the loading phase (day 15) the difference between the quantity of supplement consumed and the amount excreted indicated that on average, 30% of the CrH_2O supplement was excreted. During the maintenance phase, days 22, 36, and 50, an average excretion rate of 96, 90, and 123% of the maintenance dose was observed. These results indicate that CrH_2O supplementation was effective for increasing TCr concentration. No difference was noted between P and C groups for creatinine excretion following CrH_2O supplementation (days 15 to 50) although creatinine excretion was higher for the C compared to the P group on all days. Creatinine excretion provides a direct reflection of muscle PCr concentration (Hultman et al., 1996). Since creatinine excretion was not significantly different from the P group, results indicated that CrH_2O supplementation did not significantly increase muscle PCr concentration.

The theoretical basis for CrH_2O supplementation involves increasing muscle PCr concentration artificially to enhance the capacity of muscle to resynthesize ATP. In the cell, substrate concentration is generally equal to or less than the Km value, which is the substrate concentration needed to produce one half the maximal velocity of an enzyme-catalyzed reaction. This allows for a substantial portion of the enzyme's catalytic ability to be used while still allowing the system to react to changes in substrate concentration (Houston 1995). However, if the substrate concentration is substantially greater than the Km value the available enzyme is used efficiently but its sensitivity to changes in substrate concentration is reduced (Houston, 1995). To increase the maximum rate (Vmax) of an enzyme catalyzed reaction it is necessary to increase the enzyme and not the substrate concentration. Linossier et al., (1997) noted that following 9 weeks of cycle ergometer sprint training, creatine kinase (CK) activity remained unchanged which suggests that CK activity is not the rate limiting step during brief high-intensity exercise. Furthermore, Karlsson et al., (1972) and Houston et al., (1977) reported 15 to 18% increases in ATP concentration and no change in PCr concentration following sprint training. The results

from the present study indicated that increasing the quantity of available substrate did not result in increased performance which suggests that high energy phosphate concentration may not be the limiting factor during brief high intensity exercise.

Blood Lactate Concentration

Post-exercise blood lactate concentration changed by 4.6% for the P group and 4.2% C group following training and supplementation. However, the change in post-exercise lactate concentration was not statistically significant either over time or between groups. Sprint training has been shown to increase post-exercise blood lactate concentration (Medbo et al., 1985; Jacobs et al., 1987; Bell et al., 1988). Although post-exercise blood lactate concentration is an indicator of the contribution of anaerobic glycolysis to energy production, several factors affect its appearance in venous blood. Blood lactate concentration is affected by both the rate of production and the rate of removal from muscle. Production is influenced by factors such as: power output during exercise; duration of exercise; and, glycolytic enzyme concentration and/or activity. The appearance of lactate in venous blood results from the rate of efflux from muscle, buffering capacity, and uptake by other tissues such as the heart, liver, and muscle.

Several studies have considered how sprint training may effect the production and/or removal of lactate. Sprint training has been shown to increase non-bicarbonate buffering capacity and post-exercise blood lactate concentration (Sharp et al., 1986; Bell et al., 1988). Linossier et al. (1993) linked improvements in sprint performance with increases in PFK and LDH activity in energy production from anaerobic glycolysis after a seven week training program. Subsequent work by Linossier et al. (1997) reported significant increases in anaerobic enzyme activity following 8 weeks of training on a cycle ergometer. Training resulted in significant increases in adenylate kinase, glycogen phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase, and fructose-6-P kinase (Linossier et al., 1997). However in the same study, no changes in lactate

dehydrogenase isoenzyme 3-hydroxy-acyl-CoA dehydrogenase or citrate synthase were noted. Interestingly, improvements in sprint performance have also been shown to coincide with a decrease in type IIb and an increase in type IIa muscle fibers (Linossier et al., 1993; Allemeier et al., 1994; Linossier et al., 1997). While skeletal muscle is clearly responsive to sprint training, it remains unclear how these changes may impact post-exercise blood lactate. As noted previously, post-exercise blood lactate concentration is influenced by both the rate of production and removal from the working muscle which in turn are influenced by buffering capacity and alterations in muscle enzyme isoforms. Consequently, unless these factors are accounted for in the experimental procedures, interpretation of a change in blood lactate concentration is difficult at best.

In this study, the exercise protocol was designed to place a significant challenge on both phosphocreatine and glucose metabolism. Since the theoretical basis for creatine supplementation includes the potential to increase the energy available from phosphocreatine during repeated sprints, blood lactate concentration was of interest. Based on the urine analysis, it can be concluded that muscle TCr concentration was increased in the C group. Power outputs increased in both groups by approximately the same magnitude and the tendency for increased post-exercise blood lactate concentrations was similar in both groups. In summary, after training power output increased without a concomitant increase in blood lactate. This finding is interesting but cannot be explained for a variety of reasons alluded to above. Of more relevance to the question at hand was the observation that increasing muscle TCr apparently does not alter the glycolytic energy contribution during repeated sprints.

Effect of Sprint Training on Anaerobic Performance

Since no statistical difference existed between P and C groups, the exercise data were combined. Sprint training increased significantly peak power output by an average of 11%. Five second power output increased significantly for sprints four and five by an

average of 15 and 18% following training. Repeated 10 s power output increased significantly ($p < 0.05$) by an average of 12, 15, 21, and 25% after training for sprints two to five respectively. Previous investigations on sprint training and exercise performance have demonstrated that short training programs of less than eight weeks can improve anaerobic performance. Although previous research has reported increases in peak power by 3 to 26 %, and mean 30 s power by 4 to 12 %, the effect of training on repeated sprint performance is not well documented (Sharp et al., 1986; Bell et al., 1988; Linossier et al., 1993; Allemeier et al., 1994; Stathis et al., 1994). Results from the present study indicated that sprint training results in greater improvements in performance for each successive sprint. Data from this study indicate that sprint training alone results in modest increases in peak power, but is most effective for improving repeated sprint performance. Power output on the Monark cycle ergometer was determined by the following calculation:

$$\text{Power (W)} = \frac{\text{force x distance}}{\text{time}}$$

where:

force = the resistance applied to the flywheel

distance = is the number of flywheel revolutions per min x the distance the flywheel travels in one revolution

time = recorded in seconds

Since the resistance applied to the flywheel and the length of the sprint interval were held constant, increases in power output occurred by increasing the number of revolutions completed during each 10 s interval. Improved sprint endurance resulted in increased performance since subjects were able to maintain a higher pedal revolution rate during each sprint. Therefore, the increases in training volume resulted in increased anaerobic capacity which led to increased repeated sprint performance.

Statistical Analysis

A small effect size of 0.27 was noted when comparisons were made between 10 s anaerobic performance for P and C groups post-training. A moderate effect size of 0.56 was noted when post-training data were compared for peak power output. A large effect size of 1.23 was noted when examination of pre and post-training 10 s power outputs were compared. A power calculation based on the effect size determined for the 10 s performance data indicates that power is equal to 91.3%. Therefore, there is a 91.3% chance of detecting an effect for a sample size of 19 subjects at a significance level of $p< 0.05$. A moderate effect size of 0.53 was determined from the 10 s data obtained from sprints 3, 4, and 5 on day 22, 36, and 50 for P and C groups respectively. Based on this information, which maximizes the difference between groups, a sample size of 90 subjects per group would be necessary to obtain a significant difference for an effect size of this magnitude.

Summary

Theoretically, CrH_2O supplementation could provide the means to delay the onset of fatigue, by increasing PCr concentration resulting in increased physical performance. However, the results of this study indicate that CrH_2O supplementation did not lead to a significant improvement in performance compared to that obtained by training alone. Factors such as changes in PCr, ADP, Pi, and H^+ concentrations are interrelated in fatigue during high-intensity short-term exercise. Perhaps the augmentation of a single component does not have a significant effect on performance (Storey et al., 1974; Hibberd et al., 1985; Webb et al., 1986; Kawai et al., 1987, Nosek et al., 1987; Godt et al., 1989; Nosek et al., 1990; Fitts et al., 1994; McLester et al., 1997; Williams et al., 1997). The sprint intervals were of 10 s duration and decreased performance may be caused by either metabolite depletion or accumulation. The depletion of high-energy phosphates is linked with decreased performance during high intensity exercise since PCr is rapidly depleted while

ATP stores remained relatively unchanged (Tesch et al., 1989; Gaitanos et al., 1993; Bangsbo et al., 1994; Brooks et al., 1997). Therefore, during high-intensity short duration exercise, the maintenance of maximal exercise intensity is a contest between the rate of ATP utilization versus the rate of ATP regeneration (Brooks et al., 1997). Since PCr rephosphorylates ATP during maximal exercise, previous research has suggested that the increased muscle TCr concentration associated with CrH_2O supplementation may provide an effective ergogenic aid for the improvement of anaerobic exercise performance (Harris et al., 1992; Greenhaff et al., 1994; Balsom et al., 1995; Casey et al., 1996; Hultman et al., 1996; Vandenberghe et al., 1997).

However, the results from this study demonstrated that increasing substrate (TCr) concentration was not effective for improving sprint performance compared to the results obtained through training alone. Although a high-dose Cr loading phase increased rapidly TCr concentration, low dose supplementation has also been shown to increase TCr (Hultman et al., 1996). The high-dose loading protocol utilized in this study resulted in a large increase in urinary creatine output with relatively small increases in creatinine excretion. Results from this study suggests that a high-dose loading protocol may not be necessary since the majority of CrH_2O consumed was excreted in the urine.

If CrH_2O supplementation increases body mass through increased water retention it is important to determine if the water is retained in the intracellular or extracellular environment. If water is held in extracellular spaces then there is probably no ergogenic benefit to increased body mass. However, if the water is stored in the intracellular space then the resulting cell volumization may provide a signal for protein synthesis (Haussinger et al., 1991). An increase in cell volume triggers an anti-proteolytic effect while decreased cell volumization results in proteolysis (Haussinger et al., 1991). Therefore, if CrH_2O supplementation increases cell volume, which may be a signal for protein synthesis, future creatine and exercise research should examine the effect of supplementation on the

mechanism and site of water retention. The absorption of creatine has been shown to be enhanced when supplementation occurred in conjunction with exercise, carbohydrate consumption, and in the presence of insulin (Haughland et al., 1975; Harris et al., 1992; Green et al., 1996a, Green et al., 1996b).

In addition, the rate of glycogen storage has been shown to be accelerated by up to 300% for a two hour period immediately following exercise (Whitney et al., 1993). Therefore, it would be of interest to determine if creatine absorption could be augmented if supplementation were to occur during that period following exercise when the rate of glycogen storage is maximized. Since CrH_2O supplementation increases skeletal muscle TCr concentration, future research should also address the effects of supplementation on the enzymes associated with high energy phosphate metabolism. Due to the large variability in creatinine excretion (Heymsfield et al., 1983), future researchers would be advised to collect at least three urine samples prior to CrH_2O supplementation, for an accurate determination of baseline creatinine. Mean baseline levels can be used to determine accurately the degree of change in creatinine output following CrH_2O supplementation. In addition, daily fluid consumption should be monitored during the loading phase to determine the pattern and extent of fluid retention.

Results obtained from this study suggest that CrH_2O supplementation was not effective for increasing sprint performance compared to the results obtained through training alone. However, if individuals wish to try creatine supplementation several factors should be considered. If the athlete is participating in an activity where weight classes are used, it should be noted that supplementation resulted in a rapid increase in body mass due to increased fluid retention. The high excretion rate of creatine noted during the loading phase suggested that a high-dose loading protocol may not be required, since low-dose supplementation has also been shown to increase muscle TCr (Hultman et al., 1996). Furthermore, the absorption of creatine is enhanced when combined with exercise and

carbohydrate consumption, therefore optimal creatine absorption may occur during the two hours immediately following exercise when the action of insulin is maximized. Although, no harmful side-effects have been documented with short-term high-dose creatine supplementation, there is limited information concerning the effects of long-term high-dose use. Therefore, it would be ill-advised to continue long-term high-dose supplementation until further information is available.

Conclusion

Future research should continue to examine the morphological, health, and performance outcomes of CrH_2O supplementation in combination with various types of training. It would be of interest to investigate the mechanism(s) through which the rapid increase in body mass occurs. The present study concluded that increases in body mass for the C group were associated with decreased urinary output, and future investigations should concentrate on the magnitude and site of fluid retention. Although short-term high dose supplementation has not been shown to elicit harmful side effects the effect of long-term high dose supplementation has not been fully considered. Until this possibility is eliminated, ethical concerns dictate a conservative approach to the use of this supplement.

The present study demonstrated that CrH_2O supplementation provided no benefit during sprint training compared to the effects of training alone. Additional research utilizing different types and volumes of training is recommended. The majority of work involving CrH_2O supplementation has used a high-dose loading phase (typically 20 - 30 g/d) followed by a lower dose maintenance phase (5 g/d), or doses based on total body weight. Future work may wish to examine the effects of low dose supplementation based on a percentage of fat free mass since present results indicate that large quantities of the supplement were excreted. Many questions remain, however since in the present investigation, CrH_2O supplementation failed to enhance performance after an intense, carefully controlled training program, the efficacy of this compound must be questioned.

Bibliography

Allemeier, C.A., Fry, A.C., Johnson, P., Hikida, R.S., Hagerman, F.C., & Staron, R.S. (1994). Effects of sprint cycle training on human skeletal muscle. **J. Appl. Physiol.** 77(5): 2385-2390.

Balsom, P.D., Harridge, S.D.R., Soderlund, K., Sjodin, B. & Ekblom, B. (1993). Creatine supplementation per se does not enhance endurance exercise performance. **Acta. Physiol. Scand.** 149: 521-523.

Balsom, P.D., Soderland, K., Sjodin, B., & Ekblom, B. (1995). Skeletal muscle metabolism during short duration high-intensity exercise: influence of creatine supplementation. **Acta. Physiol. Scand.** 155: 303-310.

Balsom, P.D., Soderlund, K., & Ekblom, B. (1994). Creatine in humans with special reference to creatine supplementation. **Sports Med.** 18(4): 268-280.

Bangsbo, J., Graham, T., Johansen, L., & Saltin, B. (1994). Muscle lactate metabolism in recovery from intense exhaustive exercise: impact of light exercise. **J. Appl. Physiol.** 77(4): 1890-1895.

Bar-Or, O. (1987). The Wingate Anaerobic Test: An update on methodology, reliability, and validity. **Sports Med.** 4: 381-394.

Bell, G.J., & Wenger, H.A. (1988). The effect of one legged sprint training on intramuscular pH and nonbicarbonate buffering capacity. **Eur. J. Appl. Physiol.** 58: 158-164.

Bessman, S.P., & Greiger, P.J. (1981). Transport of energy in muscle: the phosphorylcreatine shuttle. **Science.** 211(30): 448-452.

Birch, R., Noble, D., & Greenhaff, P. L. (1994). The influence of dietary creatine supplementation on performance during repeated bouts of maximal isokinetic cycling in man. **Eur. J. Appl. Physiol.** 69: 268-270.

Bompa, T.O. (1990). Periodization of strength: the most effective methodology of strength training. **N.S.C.A. J.** 12(5): 49-52.

Brooks, G.A., Fahey, T.D., White, T.P. (1996). **Exercise Physiology: Human Bioenergetics and Its Applications**. Second Edition, Mayfield Publishing Company.

Brunner, R. (1995). Creatine use in elite powerlifting. In **Powerlifting USA** 19(2): 17.

Casey, A., Constantin-Teodosiu, S., Hultman, H.E., & Greenhaff, P.L. (1996). Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. **Am. J. Physiol.** 271(34): E-31-E37.

Cooke, R., & Pate, E. (1985). The effects of ADP and phospahte on the contraction of muscle fibers. **Biophys. J.** 48: 789-798.

Cooke, W. H., Grandjean, P. W., & Barnes, W. S. (1995). Effect of oral creatine supplementation on power output and fatigue during bicycle ergometry. **J. Appl. Physiol.** 78(2): 670-673.

Crim, M.C, Calloway, D.H., & Margen, S. (1976). Creatine metabolism in men: creatine pool size and turnover in relation to creatine intake. **J. Nutr.** 106: 371-381.

Crim, M.C, Calloway, D.H., & Margen, S. (1975). Creatine metabolism in men: urinary creatine and creatinine excretions with creatine feeding. **J. Nutr.** 105: 428-438.

Delanghe, J., De Slypere, J.P., De Beyzere, M., Robbrecht, J., Wieme, R., & Vermeulen, A. (1989). Normal reference values for creatine, creatinine, and carnitine are lower in vegetarians. **Clinical Chem.** 35(8): 1802-1803.

Earnest, C.P., Snell, P.G., Rodriguez, R., Almada., A.L., & Mitchell, T.L. (1995). The effect of creatine monohydrate ingestion on anaerobic power indices, muscular strength and body composition. **Acta Physiol. Scand.** 153: 207-209.

Evans, J.A., & Quinney, H.A. (1981). Determination of resistance settings for anaerobic power testing. **Can. J. Appl. Spt. Sci.** 6(2): 53-56.

Febbraio, M.A., Flanagan, T.R., Snow, R.J., Zhao, S. & Carey, M.F. (1995). Effect of creatine supplementation on intramuscular TCr, metabolism and performance during intermittent, supramaximal exercise in humans. **Acta Physiol. Scand.** 155, 387-395.

Fitch, C.D., & Shields, R. (1966). Creatine metabolism in skeletal muscle. **J. Biol. Chem.** 241(15): 3611-3614.

Fitts, R.H. (1994). Cellular mechanisms of muscle fatigue. **Physiol. Rev.** 74(1): 49-94.

Gaitanos, G.C., Williams, C., Boobis, L.H., & Brooks,S. (1993). Human muscle metabolism during intermittent maximal exercise. **J. Appl. Physiol.** 75(2): 712-719.

Gilpin, M.P., Bell, G., & Quinney, H.A. (1996). Modification of the wingate anaerobic power test for rowing: optimization of resistance settings. **Physiol.** 39(5): A95.

Godt, R.E., & Nosek, T.M. (1989). Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. **J. Physiol.** 412: 155-180.

Green, A.L., Hultman, E., MacDonald, I.A., Sewell, D.A., & Greenhaff, P.L. (1996)a. Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. **Am. J. Physiol.** 271(34): E821-E826.

Green, A.L., Simpson, E.J., Littlewood, J.J., MacDonald, I.A., & Greenhaff, P.L. (1996)b. Carbohydrate ingestion augments creatine retention during creatine feeding in humans. **Acta. Physiol. Scand.** 158: 195-202.

Greenhaff, P.L., Bodin, K., Harris, R.C., Hultman, E., Jones, D.A., McIntyre, D.B., Soderlund, K., & Turner, D.L. (1993). The influence of oral creatine supplementation on muscle phosphocreatine resynthesis following intense contraction in man. **J. Physiol.** 467: 75P.

Greenhaff, P.L., Casey, A., Short, A.H., Harris, R., Soderlund, K., & Hultman, E. (1993). Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man. **Clinical Sci.** 84: 565-571.

Greenhaff, P.L., Bodin, K., Soderlund, K., & Hultman, E. (1994). Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. **Am J. Physiol.** 266: E725-E730.

Greenhaff, P.L., (1995). Creatine and its application as an ergogenic aid. **Int. J. Sports Nutr.** 5: S100-S110.

Guimbal, C., Kilimann, M.W. (1993). A Na^+ dependent creatine transporter in rabbit brain, muscle, heart, and kidney. **J. Biol. Chem.** (268) 12: 8418-8421.

Harris, R. C., Soderlund, K. & Hultman, E. (1992). Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. **Clinical Sci** 83: 367-374.

Harris, R.C., Viru, M., Greenhaff, P.L., & Hultman, E. (1993). The effect of oral creatine supplementation on running performance during maximal short term exercise in man. **J. Physiol.** 467: 74P

Haughland R.B., & Chang, D.T. (1975). Insulin effect on creatine transport in skeletal muscle. **Proc Soc Exp Biol Med.** 148: 1-4.

Heymsfield, S.B., Arteaga, C., McManus, C., Smith, J., & Moffit, S. (1983). Measurement of muscle mass in humans: validity of the 24 hour urinary creatine method. **Am. J. Clinical Nutr.** 37: 478-494.

Hibberd, M.G., Dantzig, J.A., Trentham, D.R., & Goldman, Y.E. (1985). Phosphate release and force generation in skeletal muscle fibers. **Science** 228: 1317-1319.

Houston, M.E. and Thomson, J.A. (1977). The response of endurance adapted adults to intense anaerobic training. **Eur. J. Appl. Physiol.** 36, 207-213.

Houston, M.E. (1995). Biochemical energetics. In **Biochemistry Primer For Exercise Science**. Human Kinetics pg: 49-56.

Hultman, E., Soderlund, K., Timmons, J.A., Cederblad, G., & Greenhaff, P.L. (1996). Muscle creatine loading in men. **J. Appl. Physiol.** 81(1): 232-237.

Haussinger, D., & Lang, F. (1991). Cell volume in the regulation of hepatic function: a mechanism for metabolic control. **Biochimica et Biophysica Acta.** 1071: 331-350.

Karlsson, J., Nordesjo, L., Jorfeldt, L., & Saltin, B. (1972). Muscle lactate, ATP, and CP levels during exercise after physical training in man. **J. Appl. Physiol.** 33(2): 199-203.

Kawai, M., Guth, K., Winnikes, K., Haist, C., & Ruegg, J.C. (1987). The effect of inorganic phosphate on the ATP hydrolysis rate and the tension transients in chemically skinned rabbit psoas fibers. **Pflugers Arch.** 408: 1-9.

Komi, P.V. (1986). How important is neural drive for strength and power development in human skeletal muscle? In: Saltin, B. (ed.) Biochemistry of Exercise VI. Champaign: Human Kinetics Publishers Inc.

Kreider, R.B., Ferreira, M., Wilson, M., Grindstaff, P., Plisk, S., Reinardy, J., Cantler, E., & Almada, A.L. (1998). Effects of creatine supplementation on body composition, strength, and sprint performance. **Med. Sci. Sports Exerc.** 30(1): 73-82.

Linossier, M.T., Denis, C., Dormois, A., Geyssant, A., and Lacour, J.R. (1993). Ergometric and metabolic adaptation to a 5 s sprint training programme. **Eur. J. Appl. Physiol.** 67: 408-414.

Linossier, M.T., Dormois, D., Perrier, C., Frey., Geyssant, A., & Denis, C. (1997). Enzyme adaptations of human skeletal muscle during bicycle short sprint training and detraining. **Acta. Physiol. Scand.** 161: 439-445.

Loike, J.D., Somes, M., & Silverstein, S.C. (1986). Creatine uptake, metabolism, and efflux in human monocytes and macrophages. **Am. J. Physiol.** 251: C128-C135.

McLester, J.R. (1997). Muscle contraction and fatigue: the role of adenosine 5'-diphosphate and inorganic phosphate. **Sports Med.** 23(5): 287-305.

Medbo, J. I., and Burgers, S. (1990). Effect of training on the anaerobic capacity. **Med. Sci. Sports Exerc.** 22(4): 501-507.

Mujika, I., Chatard, J.C., Lacoste, L., Barale, F., & Geyssant,A. (1996). Creatine supplementation does not improve sprint performance in competitive swimmers. **Med. Sci. Sports. Exerc.** 28(11): 1435-1441.

Nosek, T.M., Fender, K.Y., & Godt, R.E. (1987). It is diprotonated inorganic phosphate that depresses force in skinned skeletal muscle fibers. **Science** 236: 191-192.

Nosek, T.M., Leal-Cardoso, J.H., McLaughlin, M., & Godt, R., E. (1990). Inhibitory influence of phosphate and arsenate on contraction of skinned skeletal and cardiac muscle. **Am. J. Physiol.** 259(28): C933-C939.

Odland, M.L., MacDougall, J., Tarnopolsky, M.A., Elorriaga, A., & Borgmann, A. (1997). Effect of oral creatine supplementation on muscle [PCr] and short-term maximum power output. **Med. Sci. Sports. Exerc.** 29(2): 216-219.

Sharp, R.L., Costill, D.L., Fink, W.J., and King, D.L. (1986). Effect of eight weeks of bicycle ergometer sprint training on human muscle buffer capacity. **Int. J. Sports Med.** 7: 13-17.

Sipila, I., Rapola, J., Simell, O., & Vannas, A. (1975). Supplementary creatine as a treatment for gyrate atrophy of the choriod and retina. **N Eng J Med.** 304 (15): 867-870.

Smith, D.J. (1987). The relationship between anaerobic power and isokinetic torque outputs. **Can. J. Spt. Sci.** 12(1): 3-5.

Stathis, C.G., Febbraio, M.A., Carey, M.F., & Snow, R.J. (1994). Influence of sprint training on human skeletal muscle purine nucleotide metabolism. **J. Appl. Physiol.** 76(4): 1802-1809.

Storey, K.B., & Hochachka, P.W. (1974). Activation of muscle glycolysis: a role for creatine phosphate in phosphofructokinase regulation. **FEBS.** 46(1): 337-339.

Stroud, M.A., Holliman, D., Bell, D., Green, A.L., MacDonald, I.A. & Greenhaff, P.L. (1994). Effect of oral creatine supplementation on respiratory gas exchange and blood lactate accumulation during steady state incremental treadmill exercise and recovery in man. **Clinical Sci.** 87: 707-710.

Tesch, P.A., Thorsson, A., & Fujitsuka, N. (1989). Creatine phosphate in fiber types of skeletal muscle before and after exhaustive exercise. **J. Appl. Physiol.** 66(4): 1756-1759.

Vandenberghe, K., Gillis, N., VanLeemputte, M., VanHecke, P., Vanstapel, F., & Hespel, P. (1996). Caffeine counteracts the ergogenic action of muscle creatine loading. **J. Appl. Physiol.** 80(2): 452-457.

Vandenberghe, K., Goris, M., Van Hecke, P., Van Leemputte, M., Vangerven, L., and Hespel, P. (1997). Long-term creatine intake is beneficial to muscle performance during resistance training. **J. Appl. Physiol.** 83(6): 2055-2070.

Vannas-Sulonen, K., Siplila, I., Vannas, A., Simell, O., & Rapola, J. (1985). Gyrate atrophy of the choroid and retina: A five year follow up of creatine supplementation. **Ophthal.** 92: 1719-1727.

Volek,J.S., & Kraemer,W.J. (1996). Creatine supplementation: Its effects on human muscular performance and body composition. **J. Strength and Cond. Res.** 10(3): 200-210.

Wahlfeld, A, W., Holz, G., & Bergmeyer, H.U. (1974). Creatinine. In **Methods of Enzymatic Analysis.** 4: 1786-1790. Academic Press, Inc.

Walker, J.B. (1961). Metabolic control of creatine biosynthesis. **J. Biol. Chem.** 236(2): 493-498.

Webb, M.R., Hibberd, M.G., Golman, Y.E., & Trentham, D.R. (1986). Oxygen exchange between Pi in the medium and water during ATP hydrolysis mediated by skinned fibers from rabbit skeletal muscle. **J. Biol. Chem.** 261(33): 15557-15564.

Williams J.H. (1997). Contractile apparatus and sarcoplasmic reticulum function: effects of fatigue, recovery, and elevated Ca^{2+}. **J. Appl. Physiol** 83(2): 444-450.

Whitney, E.N., and Rolfes, S.R. (1993). Fitness: physical activity, nutrients, and body adaptations. In **Understanding Nutrition** sixth edition. West Publishing Company.

Appendix A

Ethics Approval and Informed Consent

The study entitled, **"Effects of creatine monohydrate supplementation and training on anaerobic power and fatiguability"**

submitted by Stu Petersen/Michael Gilpin

meets the ethical standards of the Faculty of Physical Education and Recreation, University of Alberta and is, therefore, approved.

Nov 25/96
Date:

E. Jane Waktinson, Associate Dean
(Research and Graduate Studies)
Chair, Faculty Ethics Committee

INFORMED CONSENT
FOR THE RESEARCH PROJECT

EFFECTS OF CREATINE MONOHYDRATE SUPPLEMENTATION ON ANEROBIC POWER AND FATIGUABILITY FOLLOWING SIX WEEKS OF SPRINT CYCLE TRAINING

I_____ volunteer to participate in the research project conducted by Dr. S.R. Petersen and Mr. M.P. Gilpin to investigate the effects of oral creatine monohydrate supplementation on anaerobic performance. Creatine is a naturally occurring compound present in meats and fish. Creatine monohydrate is a nutritional supplement which has become popular with athletes participating in events which require high levels of strength, and power. I understand that the purpose of this study is to determine whether creatine monohydrate supplementation will improve repeated maximal sprinting performance.

I understand that the duration of this study will be eight (8) weeks, commencing with one week of pre-testing, six (6) weeks of sprint training, concluding with one week for the final performance evaluation.

The total time commitment would involve one to three hours of supervised testing and training per week at the University of Alberta Exercise Physiology Laboratory.

In order to qualify for participation in this study the volunteer:

- can not have used creatine supplements for at least 28 days prior to commencement of this study
- can not currently be engaged in active athletic competition
- must be willing to modify current training schedule until completion of this study
- the participant must be accustomed to intense physical training

Familiarization period will include 3 test sessions on separate days. Each session will consist of a warm-up, followed by a series of 5, 10 second maximal effort sprints, with 50 seconds of recovery between sprints. Testing will be performed on a Monark cycle ergometer modified for power testing.

Following the familiarization procedure subjects will be matched on the basis of sprint performance, and randomly assigned to either a loading (experimental) group or a placebo (control) group.

Experimental phase will last 6 weeks. Supervised training will occur 3 times per week. Over the course of the six week training program the number of sprints will increase from 4 to 10 sets, of 10 second sprints. Blood lactate analysis will occur during the familiarization week, following the creatine loading week, week 3, week 5, and during the

post-test period. Urinary analysis will require 24 hour urine collection from which a sample will be drawn for the determination of creatinine concentration which is representative of muscle creatine phosphate metabolism. Urine collection will occur during the familiarization week, following the creatine loading week, week 3, week 5 of the training program, and during the post-test period.

Please note that all blood extraction procedures will be carried by qualified personnel, and sterile conditions will be observed at all times in accordance to the provisions required by the University of Alberta and Occupational Health and Safety. All training and testing will be conducted at the University of Alberta Exercise Physiology Laboratory.

Side Effects: To date there has been no reports concerning any adverse effects with regards to creatine monohydrate supplementation.

Please note: Do to the intense anaerobic nature of the testing and training program some individuals may experience some muscle soreness. Additionally, individuals may initially experience some degree of nausea if unaccustomed to sprinting exercise.

Subject confidentiality will be maintained at all times. Participant data will be kept in a locked file accessable only to the investigators: Dr. S.R. Petersen, and Mr. M.P. Gilpin.

I understand that I am free to withdraw from the study at any time for any reason without prejudice, and I may ask questions at any time during the study by contacting:

Dr. Stu Petersen or Mr. Michael Gilpin

Office: 492-1026 Laboratory: 492-7394.

Signatures:

Subject_____ Date_____

Investigator_____ Date_____

Appendix B

Sprint Training and Testing Program

Table B1. Sprint Training and Testing Program

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Total Sprint Time per Week (s)
Familiarization		(1) 10/5(1)	2	(3) 10/5(1)	4	(5) 10/5(1)	6	150
Test 1	7	(8) 10/5 (2)	9	(10) 10/4(2)	11	(12) 10/3(3)	13	270
	14	(15) 10/4(3)	16	(17) 10/3(3)	18	(19) 10/4(4)	20	370
Test 2	21	(22) 10/5(3)	23	(24) 10/4(5)	25	(26) 10/3(6)	27	530
	28	(29) 10/4(6)	30	(31) 10/3(5)	32	(33)10/4(7)	34	670
Test 3	35	(36) 10/5(4)	37	(38) 10/4(8)	39	(40) 10/6(3)	41	700
	42	(43) 10/6(3)	44	(45) 10/6(4)	46	(47) 10/5(3)	48	570
Test 4	49	(50) 10/5(1)						50

Code: 10/4 (2)
Read As: 10 s sprint/4 repeats (2 sets)

Rest Interval: 50 s between repeats
5 min between sets

Resistance: 100 g/kg

Appendix C

Biochemical Assay Procedure

Biochemical Assay Procedure

The series of reactions is listed as follows:

Reaction (1) Creatinine + H_2O $\xrightarrow{\text{creatinase}}$ Creatine

Reaction (2) Creatine + ATP $\xrightarrow{\text{creatine kinase}}$ Creatine phosphate + ADP

Reaction (3) ADP + PEP $\xrightarrow{\text{pyruvate kinase}}$ ATP + Pyruvate

Reaction (4) Pyruvate + NADH + H^+ $\xrightarrow{\text{lactate dehydrogenase}}$ Lactate + NAD^+

The equilibrium constant for reaction (1) is K'H2O = 1.27 at pH 8.0 in a 0.1 M glycylglycine buffer at 37 ^oC for optimum measuring conditions. The Michaelis constant of creatininase for creatinine is very large (10^{-2} M) which is a slow reaction in a coupled assay. Increased rates can be obtained only by the addition of large amounts of creatininase with sufficient activity of auxiliary and indicator enzymes. An optimal pH range between pH 7.5 and 8.5 exist depending on whether a 0.1 M glycine or 0.1 M glycylglycine buffer is used. Triethanolamine, tris, diethanolamine, amediol and 2-amino-2-methypropanol buffers (0.1 M) displace the optimum pH into the acidic range. This series of buffers will inhibit the overall reaction, as well as buffers with a high ionic strength. Detergents such as Ultravon or Triton-X-100 are used to avoid the occurrence of turbidity in the assay mixture. The assay was conducted at 30 ^oC which is a compromise between a rapid reaction and a temperature dependent "creep". All enzyme solutions should be suspended in glycerol since the presence of ammonium ions will interfere with the reaction. In addition, ATP must be completely free from ADP, PEP, and pyruvate. All four enzymes must not contain more than 0.01 % ATPase, myokinase, hexokinase or other kinases relative to their respective activities.

Reagents

1. Glycine

2. Disodium hydrogen phosphate, $Na_2HPO_4 \cdot 12H_2O$

3. Hydrochloric Acid, HCL

4. Sodium hydroxide, NaOH

5. Triton-X-100

6. Magnesium chloride, $MgCl_2 \cdot 6H_2O$

7. Sodium hydrogen carbonate, $NaHCO_3$

8. Phosphoenolpyruvate, PEP tricyclohexylammonium salt

9. Adenosine triphosphate, ATP disodium salt, $ATP\text{-}Na_2H_2 \cdot 3H_2O$

10. Reduced nicotinamide-adenine dinucleotide, NADH disodiu n salt, $NADH\text{-}Na_2$

11. Creatine kinase, CK from rabbit muscle, lyophilized preparation ≥ 18 U/mg (25 °C)

12. Lactate deydrogenase, LDH from skeletal muscle in 50 % glycerol, pH ca 7; ≥ 550 U/mg (25 °C)

13. Pyruvate kinase, PK from rabbit muscle, solution in 50 % glycerol, pH ca. 6; ≥ 200 U/mg (25 °C)

14. Creatinine amidohydrolase (creatininase) from micro-organisms, solution in 50 % glycerol; ≥ 50 U/mg. (25 °C)

Preparation of Solutions

Solution 1: Glycine/phosphate buffer (0.1 M glycine; 0.1 M Na_2HPO_4; 0.1 % Triton-X-100; pH 8.0). Dissolve 7.51 g glycine and 35.8 g $Na_2HPO_4 \cdot 12\ H_2O$ in 800 ml distilled water, add 1 ml Triton-X-100, adjust to pH 8.0 with dilute hydrochloric acid and dilute to 1000 ml with distilled water.

Solution 2: Magnesium chloride solution (1 M): Dissolve 2.03 g $MgCl_2 \cdot 6H_2O$ with distilled water and make up to 10 ml.

Solution 3: Reduced nicotinamide-adenine dinucleotide/adenosine triphsphoenopyruvate (2 mM ß-NADH, 5.5 mM ATP, 6 mM PEP, 0.1 M glycyglycine buffer, pH 8.0): Dissolve 132 mg glycyglycine in 8.0 ml distiled water, adjust to pH 8.0 with 1 N NaOH. Add 16.6 mg ß-NADH-Na_2, 34 mg ATP-$Na_2H_2 \cdot 3H_2O$ and 28.4 mg PEP•$(CHA)_3$, check pH and dilute to 10 ml with distilled water.

Solution 4: Lactate dehydrogenase/pyruvate kinase, LDH/PK (700 U/ml; 300 U/ml): Dilute the stock suspensions accordingly with 50 % glycerol and mix.

Solution 5: Sodium bicarbonate solution (0.5 5 $NaHCO_3$): Dissolve 0.5 g $NaHCO_3$ in distilled water and dilute to 100 ml.

Solution 6: Creatine kinase, CK (1500 U/ml): Dissolve 75 mg protein in 1.0 ml 0.5 % $NaHCO_3$ (solution 5), centrifuge off any precipitate if necessary.

Solution 7: Creatinine amidohydrolase, creatininase (500 U/ml): Dilute stock solution with 50 % glycerol.

Stability of Solutions

All solutions and suspensions should be stoppered and store under refrigeration at 0 to -4 ^{o}C. Solutions 1, 2,4, 5, and 7 are stable for 1 year. Solution 3 must be prepared freshly every 14 days, and solution 6 must be prepared freshly every day.

Preparation of Samples

Urine must be collected for a 24 hour period. Subjects should maintain a normal fluid intake during the collection period and should eliminate or minimize excessive consumption of diuretic beverages such as alcohol and caffeine or exercise induced dehydration.

A 5 ml sample of urine was withdrawn and frozen for future analysis. Samples are stable indefinitely if frozen. In preparation for the assay a 0.25 ml sample was diluted 1 + 49 with doubly distilled water.

The spectrometer was set to a wavelength of 340 nm with a light path of 1cm. The water bath was set to a temperature of 30 ^{o}C, and all pipettes were calibrated prior to use.

Assay Procedure

The inclusion of an intermediary step in the creatinine assay allowed for the determination of urinary creatine and creatinine concentrations. In order to determine the concentration of both metabolites a three step procedure was established. The initial optical density was determined by first combining the glycine phosphate buffer, $MgCl_2$, NADH/ATP/PEP solutions, with the LDH/PK suspension and the sample into the cuvette. Following this procedure the aliquot is removed from the cuvette and returned to its original tube. At this point the enzyme CK is added to the original mixture which is vortexed to ensure a thorough mixing of the chemical soup and enzyme. The mixture would then

incubate in the water bath for 50 min. The addition of the enzyme creatine kinase initiated the following reaction sequence;

Reaction (2) Creatine + ATP $\xrightarrow{\text{creatine kinase}}$ Creatine phosphate + ADP

Reaction (3) ADP + PEP $\xrightarrow{\text{pyruvate kinase}}$ ATP + Pyruvate

Reaction (4) Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ Lactate + NAD^+

Following completion of the incubation period the samples were vortexed and the second optical density was recorded. The second part of this procedure required the addition of the enzyme creatininase. Following the addition of creatininase the samples were vortexed and incubated for an additional 50 min. Following the incubation period the samples were re-vortexed and the final optical density was recorded. The addition of creatininase allowed for the completion of the following series of reactions;

Reaction (1) Creatinine + H_2O $\xrightarrow{\text{creatininase}}$ Creatine

Reaction (2) Creatine + ATP $\xrightarrow{\text{creatine kinase}}$ Creatine phosphate + ADP

Reaction (3) ADP + PEP $\xrightarrow{\text{pyrvate kinase}}$ ATP + Pyruvate

Reaction (4) Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ Lactate + NAD^+

The total volume of the cuvette was made up from the following chemical proportions; glycine/phosphate buffer 1.0 ml, $MgCl_2$ solution 0.025 ml, NADH/ATP/PEP solution 0.25 ml, LDH/PK suspension, 0.025 ml, CK solution 0.05 ml, sample 0.25 ml, and creatininase solution 0.01 ml. The formula for the determination of metabolite concentration is based on the change of the optical density divided the extinction coefficient (ΔOD/6.22) results in the concentration (mM) of metabolite in the cuvette. Expanding the equation to include the total volume of the cuvette divided by the volume of sample results

in the concentration (mM) in diluted urine therefore, ΔOD/6.22 x 1.61/0.25 = [mM] in diluted urine. In order to determine the concentration of creatine in urine the ΔOD = OD1-OD2/6.22 x 1.60/0.25 x 50 = [mM] in urine. By multiplying the [mM] of creatine in the dilute sample by the volume of urine in liters equals the quantity of creatine excreted per day (mmoles/day). A similar procedure is used to determine the concentration of creatinine. For the determination of creatinine the ΔOD is the difference of OD2-OD3/6.22 X 1.61/0.25 X 50 = [mM] in urine. By multiplying the [mM] by the volume of daily urine ouput in liters results in the quantity of creatinine excretion (mmoles/day) per day. The quantity of creatine and creatinine were determined dividing the concentration of creatine and creatinine in mmoles/day by 1000 and multiplying by the molecular weight of creatine and creatinine. This calculation resulted in the determination of the quantity of creatine and creatinine excreted in g/day.

Variability of Measurement Technique

Accuracy of measurement was determined for body mass, and laboratoty pipette techniques. The standard deviation of 10 trials was used to establish a coefficicent of variability. The coefficient of variability for body mass was determined to be SD = 0.0537 kg. The coefficient of variability for pipetting technique was determined to be SD = 0.0007 g.

Appendix D

Determination of Measurement Variability

Measurement Variability

In order to determine the accuracy of measurement technique the repeatability of 10 measurement trials were performed. Variability measurements were performed for body mass and pipetting technique.

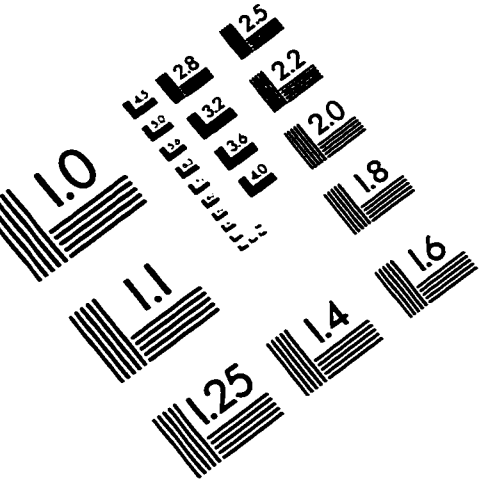
Table 1C. Variability in body mass measurement determined on balance beam scale

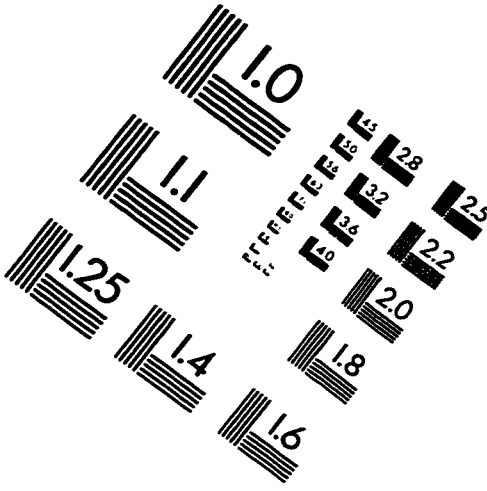
Trial	Body Mass (kg)
1	69.1
2	69.0
3	69.0
4	69.0
5	69.1
6	69.1
7	69.1
8	69.1
9	69.0
10	69.0
Mean	69.05
SD	0.05

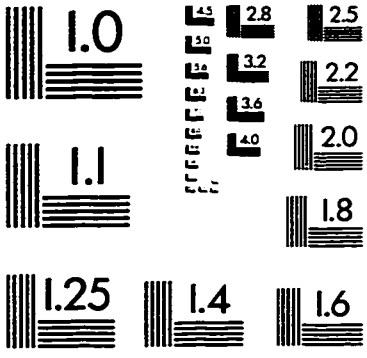
Table 2C. Variability for pipetting measurement (calibrated for 1 ml of distilled H_2O).

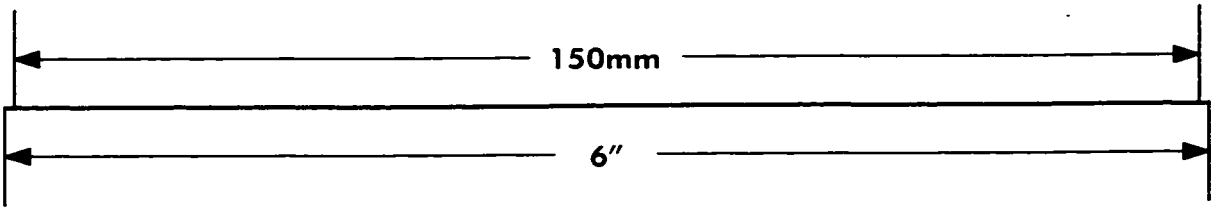
Trial	Pipette (1ml H_2O = 1 g)
1	1.000
2	0.999
3	0.999
4	1.000
5	1.000
6	1.000
7	0.999
8	0.999
9	1.001
10	0.999
Mean	0.9996
SD	0.0007

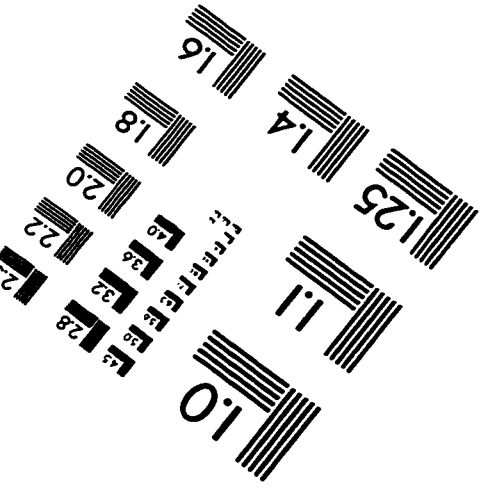
IMAGE EVALUATION
TEST TARGET (QA-3)

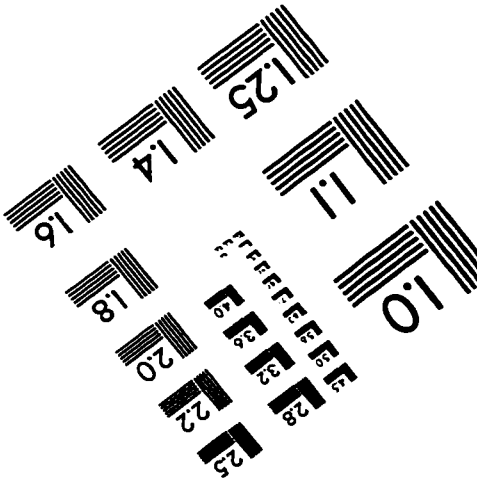












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