CREATINE AND PERFORMANCE IN MALES AND FEMALES

THE EFFECT OF CREATINE SUPPLEMENTATION ON BODY COMPOSITION, INTRAMUSCULAR PHOSPHATES AND HIGH INTENSITY EXERCISE PERFORMANCE IN MALES AND FEMALES

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ABSTRACT

Background and Rationale: Creatine phosphate (PCr) is degraded *via* the creatine kinase (CK) enzyme to provide energy to rephosphorylate ADP back to ATP during high-intensity muscle contractions. We, and others, have previously shown that dietary creatine (Cr) loading can improve performance in short-duration, high intensity exercise in males and females. An increase in fat-free mass has also been reported, which was higher for males compared to females. It was unknown whether dietary Cr loading increases intramuscular [Cr] equally in both genders. The purpose of this investigation was to examine the effect of oral Cr loading upon [PCr] and [Cr] and exercise performance in both males and females.

Methods: Twenty-seven healthy young subjects (n = 13 male, n = 14 female) participated in the investigation. Subjects performed an ischemic handgrip test, maximum voluntary contraction of dorsiflexors and knee extensors, a 30 s maximal cycling test (Wingate), and body composition (by DEXA scanning) was determined before and after supplementation with either Cr (n = 13) (5 g by mouth 4 $\bullet d^{-1}$ x 5 d, followed by 5 $g \bullet d^{-1}$ x 5 d) or placebo (pl; n = 14). Muscle biopsy samples were obtained before and after supplementation and were assayed for high-energy phosphates ([Cr], [PCr] and [ATP]).

Results: Cr supplementation resulted in an increase in intramuscular total creatine concentration [TCr] (pl, 129.9 ± 13.8 to 132.9 ± 10.8 $mMol \bullet kg^{-1}$ dw; Cr, 129.9 ± 11.6 to 146.4 ± 20.1 $mMol \bullet kg^{-1}$ dw, $p < 0.05$) and a difference in [PCr] (pl, 72.8 ± 15.2 to 67.3 ± 4.3 $mMol \bullet kg^{-1}$ dw; Cr, 70.9 ± 7.4 to 77.1 ± 13.2 $mMol \bullet kg^{-1}$ dw), but had no effect on [Cr],

and no differences in gender were observed. DEXA analysis showed no significant increase in total or fat-free mass; similarly, no improvements in maximum voluntary contractions were observed in either gender. In Wingate testing, males had a significant increase in peak power (pl, 971 ± 72 W to 989 ± 76 W versus Cr, 994 ± 174 W to 1042 ± 154 W) and peak power per kilogram of body weight (pl, 12.8 ± 1.3 to 12.9 ± 1.1 $W \bullet kg^{-1}$; Cr, 11.5 ± 1.1 to 12.1 ± 1.3 $W \bullet kg^{-1}$) as a result of creatine supplementation, while females showed no significant difference.

Conclusion: Creatine monohydrate supplementation at 20 $g \bullet d^{-1}$ for 5 days resulted in an increase in muscle total creatine concentration for males and females, but did not affect body composition or maximum voluntary contraction. Males showed improved performance in absolute and relative peak power generation with supplementation while females did not. This may suggest that males and females respond differently to Cr supplementation.

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FOREWORD

As with any research undertaking, this thesis was a collaborative effort. This investigator was solely responsible for collecting and analyzing the following:

-Performance data for handgrip, dorsiflexor fatigue protocol, isokinetic knee extension, and Wingate cycle ergometer testing,

-Blood samples for determination of lactate levels (both Dr. Tarnopolsky and Gianni Parise were responsible for the insertion of catheters for venous blood sampling.)

-DEXA scan results.

To clarify interpretation of these results, additional data need to be included. Dr. Mark Tarnopolsky was solely responsible for biopsy collection of muscle samples. Gianni Parise and Sasa Mihic were responsible for lyophilizing and powdering the muscle samples, and Mr. Parise extracted, prepared and analyzed the muscle samples for ATP, PCr and Cr with the assistance of this investigator.

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1. BACKGROUND AND STATEMENT OF PURPOSE

1.1 Introduction

There are several ways in which the body can re-phosphorylate adenosine di-phosphate (ADP) during exercise. In conditions where oxygen is available and exercise is sub-maximal, adenosine tri-phosphate (ATP) stores are replaced predominantly through the aerobic decarboxylation of fat, carbohydrate and protein. During short-term exhaustive exercise, where the demand for ATP repletion cannot be met through aerobic pathways, there are two primary anaerobic pathways. The anaerobic-lactic pathway is similar to aerobic glycolysis; however, because the aerobic capacity to metabolize pyruvate is surpassed, lactate is produced to allow glycolysis to continue anaerobically by replenishing NAD^+. The major anaerobic alactic pathway for adenosine di-phosphate (ADP) re-phosphorylation occurs via the creatine kinase (CK) -mediated phosphocreatine/creatine (PCr/Cr) pathway. To better understand the role of PCr in *in vivo* energetics, a brief outline of bioenergetic pathways will be reviewed.

1.1.1 Energetics

ATP is the currency for energy exchange in the human body. It is composed of a base, adenosine, and three inorganic phosphate molecules linked by high-energy phosphate bonds. The third high-energy phosphate group is bonded to ADP by ATPase

(complex V) enzymes in the mitochondria or by additional enzymes (e.g. CK) in the cytosol. Once the phosphorylation steps are complete, ATP is at the top of a cascade where these phosphate bonds are sequentially cleaved via ATP hydrolysis to provide energy. The first dephosphoryation releases a ΔG of -8.4 kcal/Mol (Zubay, 1995), according to the following reaction:

$$ATP + H_2O \leftrightarrow ADP + Pi + H^+ + energy \qquad \text{(Stryer, 1988)}$$

At rest, ATP hydrolysis in muscle occurs primarily to maintain homeostasis (i.e., cell repair, removal of metabolites). Additionally, ATP hydrolysis can help to restore muscle membrane potential via the ATP-dependent sodium/potassium ion pump (Stryer, 1988). Also calcium, which is released into the cytosol causing the myosin-binding site on actin to be uncovered, must be resequestered in the sarcoplasmic reticulum (via ATP-dependent ion pump, i.e. SR-Ca++ ATPase or SERCA 2) or muscle tetanus would result (Zubay, 1995). Contraction results in increased ATP hydrolysis for mechanical work in the form of actin/myosin crossbridge interaction via actomyosin ATPase (Zubay, 1995).

In times of elevated energy demand, two ADP molecules may combine to form one ATP and one adenosine monophosphate (AMP) molecule. This process is catalyzed by adenylate kinase, according to the following reaction, which has an equilibrium coefficient (k_{eq}) $\cong$ 1:

$$2\ ADP \leftrightarrow ATP + AMP \qquad \text{(Zubay, 1995)}$$

AMP can be dephosphoryated, where most is converted to uric acid through the myoadenylate deaminase pathway. The ammonia group from AMP is removed, forming

inosine monophosphate (IMP), which is converted through xanthine to uric acid. Without a shunt for AMP, the adenylate kinase reaction would quickly come to equilibrium, preventing ATP formation in this manner. A small amount of dephosphorylated AMP can yield adenosine. This adenosine base is a precursor to deoxyriboneucleic acid (DNA), riboneucleic acid (RNA) and several cofactors in enzyme kinetics.

ADP is also rephosphorylated anaerobically via the glycolytic pathway (Zubay, 1995). This is a linked set of cytosolic reactions catalyzed by 10 enzymes:

Briefly, when muscle glycogen is used as a metabolic fuel (most energy is derived from this source during exercise), it enters glycolysis as glucose 1-phosphate (G-1-P). G-1-P is converted (via phosphomutase) to glucose 6-phosphate (G-6-P). When blood glucose is used, it is imported into the cell through one of two known glucose transporters (Glut-1 and Glut-4) and phosphorylated (via ATP-dependent hexokinase) to G-6-P. G-6-P is then converted to fructose 6-phosphate and an additional phosphate group is added by phospho-fructo-kinase (also ATP-dependent). This six-carbon fructose 1,6 bis-phosphate molecule is cleaved into two three-carbon units, which are further broken down to provide energy to rephosphorylate a net equivalent of two ATP, along with carbon dioxide and H_2O. The end product of this process is pyruvate. Under aerobic conditions, pyruvate enters the tricarboxylic acid (TCA) cycle in the mitochondria to produce reduced compounds ($NADH + H^+$/ $FADH_2$), which are oxidized in the electron transport chain.

When oxygen is not readily available, or if the rate of pyruvate production exceeds the maximal rate of its oxidation, it is converted to lactate. This conversion is accomplished by the enzyme lactate dehydrogenase (LDH) in the following reaction;

$$\text{pyruvate} + NADH + H^+ \Leftrightarrow \text{lactate} + NAD^+ + H^+ \qquad \text{(Zubay, 1995)}$$

The nicotinamide adenine dinucleotide (NAD^+) regeneration is essential in order to continue glycolytic flux anaerobically. In its reduced form, NADH is unable to accept the H^+ resulting from the oxidation of glyceraldehyde 3-phosphate, halting glycolysis without the production of ATP (Zubay, 1995).

The most rapid method of rephosphorylation is via the CK reaction. This reaction is delineated by;

$$H^+ + ADP + PCr \Leftrightarrow Cr + ATP \qquad \text{(Zubay, 1995)}$$

The CK reactions involve two separate enzymatic pathways: mitochondrial creatine kinase (mtCK) phosphorylates free creatine in the mitochondria (IMM space), and cytosolic creatine kinase (cCK) removes the phosphate group in the cytosol to provide energy. While these are linked reactions, they function independently; miCK cannot phosphorylate under anaerobic conditions but cCK is able to hydrolyze PCr in the cytosol independent of oxygen. The change in free energy between phosphorylated and dephosphorylated forms of Cr is quite similar to that of ATP (ΔG = -10.4 kcal/mol, Zubay, 1995) and the CK reaction proceeds at a faster maximal rate than that catalysed by ATPases (Zubay, 1995).

In the muscle, PCr is a temporal energy buffer, rephosphorylating ADP prior to the full activation of anaerobic glycolysis during exercise (Hultman et al., 1990). The amount of PCr present for use as energy in the cytosol is related to the amount of free Cr, inorganic phosphate (P_i), and the activity of the mtCK enzyme in the intermembranous space of the mitochondria;

$$Cr + P_i \Rightarrow PCr \qquad \text{(Zubay, 1995)}$$

The interconversion of free Cr and P_i to PCr, and thus the rephosphorylation potential for ATP during exercise, is accomplished through the proposed PCr shuttle. At rest, the concentration of TCr is approximately 20 – 35 $mMol \bullet kg^{-1}$ of wet muscle (Fedosov, 1994), or 125 $mMol \bullet kg^{-1}$ dw (Harris et al., 1992). Approximately 75 $mMol \bullet kg^{-1}$ dw is found as PCr, and this concentration decreases in a relatively curvi-linear fashion, at a rate of about 2.4% per second during the first 30 s of muscular contraction (Quistroff et al., 1993). Once dephosphorylated by cCK, free Cr moves (via porin) to the mitochondria to be rephosphorylated by mtCK (Fedosov, 1994). The "phosphocreatine shuttle" will be explained more fully in section 1.3.1.1. This system is preferred to the use of adenine nucleotides (ATP and ADP) for outer mitochondrial membrane (OMM) energy transfer because of the higher diffusion permeability of guanidines (Saks et al., 1993).

During the rest-exercise transition, ATP stores would be used within seconds if it were not for the PCr system and anaerobic glycolysis. These systems provide for re-phosphorylation of ADP, although the maximum rate of the two systems is quite

different. With anaerobic gylcolysis, the maximum rate of rephosphorylation of ATP represents approximately one-third of the maximal rate of its consumption (Odland et al., 1994), and results in the formation of lactate in the working muscle (Gitanos et al., 1993). The maximal rate of ATP supply through PCr, however, closely mirrors that of ATP use. Thus, during the first 10 - 12 s of contraction, PCr provides the majority of the re-phosphorylation potential (Hultman et al., 1991).

Although PCr is the preferred fuel during the first few seconds of contraction, its rapid decrease in intramuscular concentration places a limitation on the duration of peak force production of a muscle during a maximum contraction. Beyond 3 - 5 s of contraction the maximal rate of ATP usage, and thus the maximal force, which the muscle is capable of producing can no longer be maintained. As such, the intramuscular supply of PCr could be considered a possible site of metabolic fatigue during exercise of this duration (i.e. 10-15 s (Fitts, 1994)).

1.2 Creatine Metabolism

Biosynthesis of Cr occurs primarily in the liver, and also in the kidney (Sandberg et al., 1953) and pancreas (Walker & Walker, 1959); however, Cr can also be obtained through a typical diet including meat products (Lykken, 1980). It has been found that both male and female vegetarians have a lower muscle [TCr] (Delanghe et al., 1989). Endogenous synthesis of Cr occurs via a two-step process. Amidinotransferase is the first

and rate-limiting step that joins arginine and glycine. N-methlytransferase delivers a methyl group from S-adenosylmethionine to form the di-peptide Cr (Walker, 1979).

Eighty-five percent of total Cr is found intramuscularly, on average with 40 percent as free Cr, and the remaining 60 percent in the phosphorylated form (Heymsfield et al., 1983; Greenhaff et al., 1996). Because muscle is unable to manufacture Cr, transport and uptake of this guanidine are vital. Cr is carried in the plasma, and is transported into muscle primarily through a membrane-bound sodium-dependent symport (Loike et al., 1986). Indeed, approximately 80% of Cr-transport was found to occur through the sodium-dependent symport in muscle (rat soleus) (Willott et al., 1999). A second, lower volume transporter is also present, and is choride-dependent (Guimbal & Kilimann, 1993).

Transport of Cr into muscle is stimulated by physiologically high levels of insulin (Steenge et al., 1998) and inhibited with decreases in sodium (Willott et al., 1999) and vitamin E (Gerber et al., 1962). The performance of sub-maximal exercise (Harris et al., 1992) and the ingestion of carbohydrate (Green et al., 1996) also enhance Cr absorption.

The majority of Cr loss from muscle under normal physiological conditions occurs through non-enzymatic conversion to creatinine (Walker, 1979). With Cr supplementation, where supra-physiological levels of plasma Cr are present, it has recently been shown that the majority of loss is through direct urinary excretion (VanDenBerghe et al., 1997). Both Cr and creatinine are excreted in urine; however, some Cr may be recycled by retention at the kidney (Walker, 1979).

1.3 Creatine as an ergogenic aid

As early as the mid-sixties, interest in Cr metabolism has been a focus of research studies (Fitch et al., 1968). In 1977, MacDougall et al. demonstrated that 5 months of weight training in previously untrained male subjects resulted in a 39% increase in intramuscular [TCr]. While this observation has not been duplicated to the same extent in any recent experimentation MacDougall et al. (1977) used muscle homogenate and the increase in [TCr] was likely due to preferential hypertrophy of type II muscle fibers which have greater [TCr] than type I), the result led to speculation that this physiological adaptation may have an effect in improving performance in muscular strength and power. In the last decade there has been a resurgence of interest in Cr metabolism/supplementation in humans, based largely upon findings of Harris et al. (1992), when Cr supplementation was shown to increase the intramuscular TCr pool in man.

Effective Cr supplementation protocols (where muscular [TCr] increases by >20 $g \bullet kg^{-1}$ (Casey et al., 1996)) typically include a 3 - 7 d loading phase, where subjects consume 15-25 g of Cr in 4-10 g doses, several times each day (Juhn and Tarnopolsky, 1998a). Most commonly, Cr supplementation studies examining performance have used 5 g by mouth 4 $times \bullet d^{-1}$ for 4-5 d. Several studies have demonstrated that this protocol can increase intramuscular [TCr] stores (Harris et al., 1992, Greenhaff et al., 1994, Odland et al., 1994, Febbraio et al., 1995, Hultman et al., 1996, and Volek et al., 1996). In studies involving a loading phase longer than 4 - 5 d, muscle [Cr] reaches an apparent

maximum, with a subject-dependant range of 120 – 170 $mMol \bullet kg^{-1}$ muscle (dw) (Greenhaff et al., 1994). At this point, the appearance of Cr and creatinine in urine approximates the amount of Cr consumed (VanDenBerghe et al., 1997).

A lower dosage (maintenance phase) has been successfully employed in studies of longer duration, where subjects ingest only enough Cr to match, or slightly exceed, the amount which appears as creatinine in urine. In the few existing longitudinal Cr supplementation studies (VanDenBerghe et al., 1997, Kreider et al., 1998) Cr is ingested for the duration of the supplementation protocol at 3-5 $g \bullet d^{-1}$. A dosage as small as 2 $g \bullet d^{-1}$ has been shown to be effective in maintaining increases in muscular [TCr] typically associated with an effective loading phase (Hultman et al., 1996). For a comprehensive review see Juhn and Tarnopolsky (1998a).

The increase in muscular [PCr] has been suggested to provide a larger temporal buffer of ATP hydrolysis during exercise, and an increase in [TCr] may be responsible for an increased rate of PCr resynthesis during recovery from exercise (Greenhaff et al., 1996). The degree to which the TCr pool is enlarged is however highly variable, both among subjects and between experiments (Greenhaff et al., 1994; Harris et al., 1992, Odland et al., 1997).

As previously mentioned, it has been suggested that there is a threshold level of increase in intramuscular Cr concentration necessary for an improvement in the performance of anaerobic exercise during supplementation (Greenhaff et al., 1994; Casey et al., 1996). In these experiments, subjects who showed an increase in TCr

concentration of greater than 20-mMol•kg^{-1} dw demonstrated an improvement in short-term exhaustive exercise (Casey et al., 1996). Several possible reasons for the variability of response to Cr loading have been mentioned. Ingestion of carbohydrate (Green et al., 1996), performance of exercise during the supplementation period (Harris et al., 1992) and having a low initial TCr concentration prior to supplementation (Greenhaff, 1994) all appear to increase the potential to load.

1.3.1 Exercise

There is still some controversy regarding the overall efficacy of Cr supplementation, as observed effects on performance have been highly variable. Some researchers have found increases in high intensity performance (Greenhaff et al., 1993a; Balsom et al., 1995; Earnest et al., 1995; Bosco et al., 1997; Jacobs et al., 1997; Kreider et al., 1998 and others), while others have not (Balsom et al., 1993; Odland et al., 1994; Dawson et al., 1995; Febbraio et al., 1995; Terrillion et al., 1997; Snow et al., 1998 and others). Still others have found significant weight gains with supplementation (Balsom et al., 1993; Barnett et al., 1996; Cooke and Barnes, 1997; Dawson et al., 1995; Earnest et al., 1995; Greenhaff et al., 1994; Volek et al., 1997) and a decrease in dynamic aerobic performance due to this (Balsom et al., 1993). In certain sports the increase in weight may even be detrimental to exercise performance, limiting maximal achievement in

weight-bearing exercises. Conversely, the weight gain may be indicative of an anabolic effect of Cr supplementation (Kreider et al., 1998).

Additionally, Cr supplementation may have an effect on lactate production during exercise. Some researchers have discovered an increase during Cr feeding (Meyer et al., 1986; Tarnopolsky et al., 1997), while others have found an attenuation of plasma lactate accumulation when exercise was performed during the supplementation period (Balsom et al., 1995; Volek et al., 1996). An examination of intramuscular lactate production (a more direct measure than that of blood) in Cr-fed males showed a significant increase. Pre-supplementation values obtained immediately post-muscular contraction were 95.7 ± 8.3 and increased to 110.3 ± 5.0 $mMol \bullet kg^{-1}$ dw with Cr supplementation (an increase of ≈ 15%) (Greenhaff et al., 1994).

Volek and Kraemer (1996) have suggested that when the CK reaction is in favor of ATP regeneration, PCr provides a proton-buffering effect, thereby reducing the concentration of protons generated by lactate production. However if PCr buffers H+, it may push the lactate dehydrogenase (LDH) reaction in the direction of lactate production by removing the proton (Stryer, 1988). Meyer et al. (1986) demonstrated that free Cr is required to maximally activate glycolysis, indicating that there may be a link between [PCr], [Cr] and lactate production

The effect is complex, such that there are two demonstrated roles of Cr in glycolytic flux; one states that PCr attenuates PFK activity, thus reducing the production of lactate; the other asserts that free Cr upregulates PFK (Storey et al., 1974). Although

these effects are representative of typical negative-feedback enzymatic control, varying interpretations of the data have rendered opposite conclusions regarding the effect of Cr on lactate production.

Despite varied conclusions, there is evidence supporting beneficial influences of Cr in short-term, high-intensity exercise performance (for review, see Juhn and Tarnopolsky, 1998a). Research exploring the effects of Cr supplementation on performance can be grouped based on four main theories: 1.) Cr supplementation may increase [TCr] levels (Harris et al., 1992), providing a larger spatial energy buffer (PCr shuttle), thereby improving performance in single bouts of maximal effort of duration greater than or equal to 60 s. 2.) Cr supplementation may increase [PCr] available at the onset of exercise (Harris et al., 1992), which provides a larger temporal energy buffer and may result in increased performance during single bouts of short-duration ($\approx$ 30 s) maximal activity or allow completion of a greater number of repetitions at a given resistance in weight lifting (Earnest et al., 1995). 3.) Supplementation may also increase re-synthesis of [PCr] following a bout of exercise (Greenhaff et al., 1996) and improvements in repeated bouts of maximal effort may result. 4.) Cr supplementation may increase contractile protein fractional synthetic rate or muscle protein balance through either direct or indirect routes (Ingwall et al., 1974; Haussinger et al., 1993). What follows is a review of the various theories that explore how Cr supplementation may improve performance, and experiments that have examined each of these

mechanisms. The sections are ordered by mechanism, including theory, relevant experimentation and conclusions in each section for clarity.

1.3.1.1 Maximal Exercise ≥ 60 s

The phosphocreatine shuttle (Fedosov et al., 1994) may have a role in ATP supply during aerobic exercise and aerobic recovery from anaerobic bouts. In the mitochondria, reduced nicotinamide and flavin ($NADH + H^+$ and $FADH_2$) and oxygen are used to generate ATP through oxidative phosphorylation. Newly formed ATP is transported through the inner mitochondrial membrane via the adenine neucleotide translocator (ANT). In the intermembranous space, mtCK catalyzes the transfer of the high-energy phosphate bond to creatine, which is transported through the outer mitochondrial membrane into the cytosol by porin. Once in the cytosol, PCr rephosphorylates ADP via the cCK enzyme. ATP then supplies working muscle with energy via the activity of various ATPases.

Given that an increase in total and cytosolic [Cr] is commonly observed with supplementation (Harris et al., 1992), mtCK may be stimulated, which may in turn stimulate oxidative phosphorylation. This could lead to increases in aerobic performance and may also increase PCr resynthesis during recovery periods (Bessman and Greiger, 1981, Greenhaff et al., 1993b - see section 1.3.1.3).

In light of these speculations, several researchers have conducted experiments in sub-maximal, aerobic or anaerobic exercise (for summary, see Table 1.1). In 1993, Balsom et al. had subjects exercise both on a treadmill to exhaustion at 120% of VO_{2max} and on a 6-km trail before and after Cr supplementation (or placebo control). Cr supplementation had no effect on the treadmill run or on VO_{2max}. Exhaustion on the treadmill occurred between 3 and 6 min of running. In the trail run, Cr-supplemented subjects were on average 26 s slower (significant) over the 6 kilometers. Also, Terrillion et al. (1997) found no improvement in time to run 700 m in Cr-supplemented competitive runners. These runners performed 2 x 700 m runs with a 60min recovery period, and each run was between 1.5 and 2 min in duration.

Using a treadmill set at 20 km/h with a 5° incline, Bosco and colleagues (1997) ran participants to exhaustion in approximately 1 min. The Cr-supplemented subjects showed a significant 13% improvement in time to exhaustion. Similarly, Jacobs et al. (1997) had subjects cycle to exhaustion at 125% of $VO_{2max.}$ The Cr-supplemented subjects demonstrated a significant 9% increase in time to exhaustion and also a 10% increase (significant) in maximal accumulated oxygen deficit (MAOD) during their average of 2 – 2.5 min of cycling.

Table 1.1: Studies on maximal activity of ≥60 s duration

Author	N	Testing	Results
Balsom, 1993	18♂	Running -treadmill run to fatigue at 120% VO_{2max} (3-6 min.)	No Δ in treadmill run
		-6km trail run (20-26min)	1.8% (26s) slower in trail run
		-1 day between runs	
Bosco, 1997	8♂	Running -10km/hour on treadmill for 6 min. periods (50-90% of VO_{2max})	No Δ in; O_2 consumption, respiratory exchange rate, lactate accumulation during exercise or up to 15 min of recovery
		-20km/h, 5° incline to fatigue (≈ 60s)	13% ↑ in time to fatigue
Febbraio, 1995	6♂	Cycle erg. -4x60s bouts, 1 min. recovery, fifth bout to fatigue	↑ [TCr] but no in time to exhaustion
Jacobs, 1997	21♂ 5♀	Cycle erg. -to exhaustion at 125% VO_{2max} (130-140s)	10% ↑ in "maximum accumulated oxygen deficit" 9% ↑ in time to exhaustion
Terillion, 1997	12♂	Running -2x700m (90-120s) -60 min. recovery	No Δ in running times

It is commonly observed that Cr supplementation is not effective in exercise of 3 min or more in duration. As exercise times to exhaustion decrease and approach 1 min, results showing an ergogenic effect tend to become more evident. There are several hypotheses for this observation; first, the fact that PCr has only small contributions to aerobic metabolism (Meyer et al., 1986), having achieved a concentration of ≈ 0 as maximal exercise nears the 1-min mark. Indeed, Cr has been shown to be 70% depleted after 10 s of maximal exercise, and nearly 100% depleted after 20 s (Hultman et al., 1991). The weight gain due to supplementation may also play a role as this would serve

to lower relative VO_{2max}, producing an ergolytic effect in dynamic exercise. Finally, it is very likely that the "PCr shuttle" (Fedosov et al., 1981) is either not the rate limiting step in aerobic ATP supply, or is not altered by Cr supplementation.

Possibly the most convincing argument against Cr supplementation improving aerobic metabolism exists in a study involving patients with mitochondrial cytopathies. This disease type is associated with severely depressed levels of [PCr] both at rest and post-activity and very low levels of aerobic fitness. Tarnopolsky et al. (1997) administered Cr to 7 such patients, and while indices of anaerobic performance were significantly improved, performance of lower intensity aerobic activity was unaffected.

1.3.1.2 Single Bouts of Maximal Exercise

Additional Cr supplementation research has based experimentation on the premise that by increasing the amount of PCr available at the onset of maximal exercise, performance over the first few seconds should be improved. This hypothesis stems from the observation that Cr and PCr exist in equilibrium in the cytosol. cCK is a reversible enzymatic pathway, which achieves equilibrium in resting muscle (Bessman and Geiger, 1981) where PCr represents approximately 60% of the total cytosolic creatine content (Greenhaff et al., 1994). By having more PCr present at onset of exercise, the buffering effect on ATP will continue for a longer period, theoretically enhancing performance in brief, maximal efforts (for summary, see Table 1.2).

Table 1.2: Studies on single bouts of maximal activity

Author	N	Testing	Results
Cooke, 1995	12♂	Cycle erg. -2x15s sprint -20 min. recovery	No Δ in performance
Dawson, 1995	18♂	Cycle erg. -1x10s	No Δ in performance
Odland, 1997	9♂	Cycle erg. -1x30s	No Δ in performance
Snow, 1998	8♂	Cycle erg. -1x20s	No Δ in performance

To test this hypothesis, Cooke and colleagues (1995) tested 12 male subjects on 15 s maximal bouts of cycle ergometry. Subjects performed 2 bouts during each testing session, but the bouts were separated by 20 min of recovery. No improvement in power, work or fatigue index was found with supplementation. Odland et al., (1997) and Snow et al., (1998) tested single bouts of maximal cycle ergometry of 30 s and 20 s in duration respectively, and also found no improvement in power produced following Cr loading.

Despite the lack of ergogenic effect found in the above studies, it is important to note that several of the experiments testing repeated bouts of maximal exercise did show improvements in the first exercise bout with Cr supplementation (Birch et al., 1994; Dawson et al., 1995; Earnest et al., 1995; MacLennan and Tarnopolsky, 2000; Prevost et al., 1997; Kreider et al., 1998). It is apparent that the effect of Cr supplementation on single maximal efforts is largely inconclusive, and that more research is needed.

Among criticisms of the above research, the most common argument against supplementation, whether improvement is realized or not, concerns the fact that most studies which have demonstrated improved performance have dealt only with exercise in

laboratory conditions. In this setting, it is difficult to extrapolate the effect of a commonly observed weight gain on dynamic exercise or performance in athletic activity. Thus, despite improvements in anaerobic variables, one can only speculate on the result of any application to the sporting world, which is where one is most likely to see widespread use of the supplement. It is interesting to note, however, that observed improvements in muscular force development could result in an increase in training volume when performing resistance exercise (Earnest et al., 1995; Baechle, 1994).

1.3.1.3 Repeated Bouts of Maximal Exercise

In keeping with the hypothesis that Cr supplementation may increase PCr resynthesis during recovery from exercise (Greenhaff et al., 1993b), several groups of researchers have conducted experiments on repeated bouts of maximal effort of varying length with varying recovery periods (for summary, see Table 1.3a and Table 1.3b). Birch and colleagues (1994) tested 14 male subjects on performance of 3 x 30 s bouts of maximal cycling with a 4-min recovery between bouts. In the first bout increases of greater than 5% in peak and mean power output as well as total work performed were observed in Cr-supplemented subjects (significant). In bout two, mean power and total work were improved by the same magnitude, and no increases were observed in bout 3.

Table 1.3a: Studies on repeated bouts of maximal activity showing positive effects of Cr supplementation

Author	N	Testing	Results
Birch, 1994	14♂	Cycle erg. -3x30s bouts -4 min recovery	6%↑ in mean power output and total work performed in bouts 1 & 2; no Δ in 3 8%↑ in peak power, bout 1 only
Bosco, 1997	6♂	Jump (ht) -5s cont. jumping	No Δ in performance
		-45s cont. jumping	7%↑ in height (1st 15s) 13%↑ (2nd 15s) No Δ (3rd 15s) No Δ in average power output
Dawson, 1995	22♂	Cycle erg. -6x6s bouts -24s recovery	4.6%↑ in peak power in bout 1 No Δ in bouts 2-6 4.5%↑ in total work
Earnest, 1995	8♂	Cycle erg. -3x30s bouts -5 min. recovery	13%↑ in total anaerobic work in bout1 18%↑ in bouts 2 and 3
Kreider, 1998	25♂	Cycle erg. -12x6s bouts -5min. recovery	↑ total work, bouts 1-5
Prevost, 1997	10♂ 8♀	Cycle erg. -each to exhaustion 1. continuous cycling 2. 30s:60s work:rest 3. 20s:40s 4. 10s:20s	1. 23.5%↑ in time to exhaustion 2. 61.0%↑ 3. 61.9%↑ 4. >100%↑
Volek, 1997	14♂	Jump-squat -5x10 jumps with 30% 1RM -2min. recovery	4%↑ in peak power in all sets

Table 1.3b: Studies on repeated bouts of maximal activity showing no effects of Cr supplementation

Author	N	Testing	Results
Barnett, 1996	17♂	Cycle erg. -7x10s bouts -30s recovery (except 5 min b/w bout 5-6)	No Δ in -peak power -mean power -VO_{2max}
Cooke, 1997	80♂	Cycle erg. -2x6s bouts -30,60,90,120s (variable) recovery	No Δ in performance (peak power and time to fatigue)
Deutekom, 2000	23♂	Cycle erg. -2x30s bouts -4 min. recovery	No Δ in performance
Redondo, 1996	8♂, 14♀	Sprinting -3x60m (7-8s) -2 min. recovery	No Δ in performance

In a similar cycle ergometry study, using 5 min recovery periods (Earnest et al., 1995), total anaerobic work was measured. Improvements of greater than 10% were apparent in all three bouts in the Cr-supplemented group (significant). More recently, Kreider and colleagues (1998) used 12 • 6 s bouts of cycle ergometry with 60 s recovery periods in 25 college football players, and found significant increases in total work performed in the first 5 bouts of cycling with Cr supplementation. All subjects in Cr or Pl engaged in 28 d of strength training during the supplementation period.

There are some findings that suggest Cr supplementation does not benefit performance of repeated bouts of maximal exercise (Barnett et al., 1996; Cooke et al., 1997; Redondo et al., 1996). Barnett et al. (1996) and Cooke and Barnes (1997) found no effect of supplementation on cycle ergometry; however, they used 10 s (or shorter) maximum

efforts. This duration of maximal cycling may not have been long enough to deplete normal PCr stores (likely depleted in 20-30 s). This would make it difficult to find a difference in performance even if supplementation did increase [PCr], which is uncertain since no intramuscular measures were obtained in this study. Further, several researchers have found significant increases in performance using < 10 s bouts of cycle ergometry (Dawson et al., 1995; Kreider et al., 1998; Prevost et al., 1997).

With weight-bearing or dynamic performance, Redondo et al. (1996) failed to find a difference in repeated sprint times in 9 Cr-supplemented subjects compared to placebo. It was suggested that the weight gain associated with supplementation might have negated any performance increases resulting from Cr consumption. But again, the duration of maximal effort may not have been sufficient to deplete intramuscular [PCr] (only 7-8 s). Regardless, in other research using repeated efforts of weight-bearing activity (Bosco et al., 1997; Volek et al., 1997) an ergogenic effect was demonstrated.

Experimental evidence suggests that Cr supplementation may increase performance in repeated bouts of maximal exercise, irrespective of duration of maximal effort or amount of recovery time. When these results are applied to strength training, they indicate that Cr-supplemented individuals may be able to elicit a greater training stimulus through increased training volume. It is therefore a plausible hypothesis that Cr may also have an anabolic role, albeit indirect.

1.3.1.4 Weight Training

It is likely that Cr supplementation enhances the PCr mediated temporal buffering of ATP during the initial seconds of muscle contraction. Also, Odland et al. (1994) have demonstrated that ADP rephosphorylation via PCr is equal to the rate of ATP use. It seems a logical extension to hypothesize that the Cr-supplemented muscle would be able to maintain maximal force production over a longer period of time, or for a greater number of contractions. Using a resistance-training paradigm, this hypothesis could translate into the performance of a greater number of repetitions at a given resistance, thereby increasing training volume of working contractile tissues.

Experimental evidence supporting the application of Cr supplementation to weightlifting began to surface in 1995 (for summary, see Table 1.4). Earnest et al. (1995) found a 6% increase in 1RM in bench press, along with a 30% absolute (29% relative to body weight) increase in total volume of weight lifted in a training session after 28 days of resistance training coupled with Cr supplementation.

Table 1.4: Studies on weight training

Author	N	Testing	Results
Becque, 2000*	23♂	Weightlifting -1RM arm flexion	8%↑ in 1RM post-training
Earnest, 1995	8♂	Weightlifting -70% 1RM, # reps to fatigue	26%↑ in # reps to fatigue
		-1RM bench press	6%↑ in 1RM
Kreider, 1998*	25♂	Weightlifting -weight x reps to failure on bench press, squat and power clean	225kg↑ total bench press volume, 453kg↑ total sum of bench, squat and clean
VanDenBerghe, 1997*	19♀	Weightlifting -intermittent arm flexion	10-25%↑ in intermittent exercise capacity of arm flexors
		Weightlifting -1RM on leg press, leg extension, leg curl squat and shoulder press	20-25% ↑ in leg press, leg extension and squat $p<0.15$ ↑ in bench press and leg curl No ↑ in shoulder press
Volek, 1999*	14♂	Weightlifting -5 sets to fatigue With 10RM weight -2min. recovery	1-2.3 more lifts in all sets

* indicates training study

VanDenBerghe et al. (1997) studied 19 sedentary women who were matched and randomly assigned to either Cr (n = 10) or placebo (n = 9) groups (supplementation = 4 d @ 4x5 $g \bullet d^{-1}$ followed by 10 weeks @ 5 $g \bullet d^{-1}$). Subjects trained 3 $h \bullet week^{-1}$ on leg press, squat, leg extension, leg curl, bench-press and shoulder press. 1RM on each exercise was increased, yet Cr-supplemented subjects had significantly greater improvements (20-25%) in leg press, squat, and leg extension. Power production in repeated arm flexor contractions (5 sets, 30 reps, 2 min between sets) was also increased to a greater extent after 10 weeks of training and Cr supplementation (11-25%, sets 1-5 respectively). Fat-

free mass was also increased by a significantly greater amount in the Cr-supplemented group, 5.8% versus 3.7% with placebo.

Kreider et al (1998) found similar changes in 25 college football players fed Cr (n = 11) or placebo (n = 14) for 28 d during off-season resistance/agility training. DEXA scans revealed greater increases in body mass (2.4 vs. 0.9 kg) and fat-free mass (2.4 vs. 1.3 kg) with Cr supplementation. Total training volume (volume = sets • repetitions for each exercise) in bench-press, squat and power clean was also found to increase by a significantly greater amount during training and Cr feeding (1558 (Cr) vs. 1105 (pl) kg), with the greatest increase observed in bench-press volume (225 (Cr) vs. –9 (pl) kg).

Volek et al. (1999) have recently provided further evidence. Previously strength-trained men (n=19) were supplemented with Cr (n=10; 25 $g \bullet d^{-1}$ load for 1 week, 11 weeks of 5 $g \bullet d^{-1}$) or placebo (cellulose, n=9) over 12 weeks of periodized resistance training. Increases in body mass and fat-free mass were greater with Cr supplementation (6.3% in each (Cr) vs. 3.6 and 3.1% for placebo, respectively). Increases in 1RM were also greater in the Cr group; bench-press improved by 24% (vs. 16%), while squat was enhanced by 32% (vs. 24%) during the training protocol. Further, training volume, expressed as total amount of weight lifted during a workout (sets • repetitions • weight), was greater during weeks 5-8 of training in the Cr-supplemented group.

The increase in training volume observed in the above studies could be important in increasing net muscle protein synthesis (MPS) by stimulating the fractional synthetic rate (FSR) of the exercised muscle (Biolo et al., 1995 & 1997; Chesley et al., 1992;

Phillips et al., 1997; Tipton et al., 1996 & 1999). FSR in muscle has been shown to increase acutely following resistance exercise (Biolo et al., 1995; Chesley et al., 1992; Phillips et al., 1997); however, the net muscle protein balance in Biolo et al. (1995) and Phillips et al. (1997) was found to be negative (Chesley et al. (1992) did not measure protein breakdown). This is an expected finding with fasted subjects because, despite increases in FSR, fractional breakdown rate (FBR) of muscle protein was also increased.

In fed (physiological levels of amino acids) men, Biolo et al., (1997) found a positive muscle protein balance both at rest and after strenuous exercise. The increase in MPS was attributed to large increases in FSR and an attenuation of FBR. Tipton and colleagues (1999) also found significantly improved net muscle protein synthesis in fed subjects post-resistance exercise, however both men and women were tested in this study.

If Cr induces an increase in training volume for weight lifters, then there may be a cumulative effect upon muscle via incidental increases in FSR over time. Further, in the case of fed subjects, muscle protein balance had been shown to be positive; thus, an anabolic stimulus is present.

Incidentally, training volume increases may also elicit an increase in the production of anabolic hormones, as observed in Gotshalk et al., (1997). Gotshalk and colleagues (1997) showed significant increases in serum testosterone and growth hormone in a 3 set compared to a 1 set workout in 8 recreationally trained men. Resultant gains in contractile tissue may account for some of the lean body mass increase observed during Cr supplementation (Poortmans and Francaux, 1999), but most likely only in cases

where supplementation and strength training continue for periods of a minimum 6-8 weeks (Hickson et al., 1994).

Recent experimentation has also shown that Cr supplementation resulted in a significant increase in satellite cell mitotic activity during compensatory hypertrophy in rat muscle when compared to compensatory hypertrophy alone (Dangott et al., 2000). This finding suggests that potential improvements in contractile protein synthesis with Cr supplementation may involve hyperplasia as well as hypertrophy.

1.3.2 Additional sites of protein regulation

An increase in body mass has been commonly observed during Cr-supplementation (Balsom et al., 1993; Greenhaff et al., 1994; Earnest et al., 1995; Kreider et al., 1998 and others). In short-term supplementation studies, weight gain is thought to be primarily water gain in muscle cells (Hultman et al., 1996). In studies with supplementation protocols of longer duration coupled with resistance training (e.g. Kreider et al., 1998), Cr has been shown to result in a greater increase in fat-free mass as compared to placebo, which may be indicative of gains in contractile tissue. These observations have lead to speculation regarding a possible role of Cr in protein regulation, either by direct influence (e.g., Ingwall et al., 1974), or by allowing an athlete to complete a greater number of repetitions in a given workout (e.g., Kreider et al., 1998).

As mentioned, there have been suggestions that there is likely a greater net protein synthesis among subjects who resistance train while supplementing with Cr than among subjects who resistance train without supplementation (Volek and Kreider, 1997). In addition to providing an increased training stimulus, there are several other hypotheses for a potential mechanism or loci of effect for this enhanced hypertrophy.

1.3.2.1 Direct synthetic control

Studies by Ingwall and colleagues (1974, 1976) provide additional evidence for a role of Cr in protein synthesis using muscle tissue in culture. Using controlled *in vitro* experiments, the addition of Cr to muscle cell tissue cultures yielded an increase in the synthesis of myosin heavy chain and actin proteins, which are major contractile proteins (Ingwall et al., 1974; Ingwall, 1976). In addition to showing increased muscle-specific protein synthesis, these researchers observed that Cr had no effect on protein degradation. They speculated that the effect was pre-translational based upon observed increases in mRNA. Ingwall et al. (1974) offered several possible mechanisms; Cr may either modify tRNA or amino acid pools necessary for protein synthesis, or act as a factor for transcription.

Gyrate atrophy patients exhibit a progressive degradation of fast glycolytic fibers (type IIb), which along with other symptoms, leads to a reduction in visual field (Vannas-Sulonen et al., 1985). The cause of this disease stems from an inborn error of metabolism

that results in reduced ornithine aminotransferase activity. This leads to elevated plasma ornithine levels that in turn inhibits the amidinotransferase- mediated joining of arginine and glycine, which is the rate- limiting step in the endogenous synthesis of Cr (Walker, 1979). The resultant decrease in the endogenous [Cr] is suggested to be the probable cause of type IIb skeletal muscle fiber atrophy (Vannas-Sulonen et al., 1985). In a five-year study of the effect of Cr supplementation on gyrate atrophy patients, subjects showed attenuated or reversed type IIb fiber atrophy post-supplementation when compared to the pre-supplementation values (Vannas-Sulonen et al., 1985).

Regardless of the mechanism, any combination of attenuated proteolysis or enhanced protein synthesis would result in a gain in lean body mass, providing that nitrogen balance remain positive. This would in turn relate back to increased performance in maximal anaerobic activities simply because a greater cross-sectional area of muscle is capable of generating greater force (Astrand, 1986)

1.3.2.2 Cellular hydration

A possible mechanism for enhanced contractile tissue gains as a result of Cr supplementation involves a retention of water in muscle cells, resulting in an increase in cell volume and an increase in net protein balance (Hultman et al., 1996; Oopik et al., 1998). This effect is caused by an increased intracellular concentration of a variety of compounds (see Haussinger et al., 1994), including Cr (Haussinger et al., 1993).

Groundbreaking research in the area of the relationship between cellular hydration and protein metabolism began in the early 1990's. Haussinger et al. (1990) observed that a hypoosmotically-induced increase in hepatic cell volume resulted in proteolytic inhibition in rat liver cells. Since that observation, research has been performed to understand what mechanisms result in enhanced cellular hydration, and what effect these alterations have on protein metabolism.

In vitro, hypoosmotic solutions (Stoll et al., 1992) and infusion of several osmotically active substances (Hallbrucker et al., 1991; Millar et al., 1997; also see Haussinger et al., 1994) have resulted in cell swelling and positive changes in protein metabolism. Further, Volkl et al., (1994) has shown that cell swelling results in the alkalization of lysosomes. Although lysosomal activity plays only a small role in protein breakdown (tertiary to calpain and ubiquitin pathways), it is hypothesized that alkalization would result in a decrease in autophagic activity, and a consequent reduction in proteolysis (Volkl et al., 1994).

Hallbrucker and colleagues (1991) established a linear relationship between rat liver mass increases (as a result of cell swelling due to hypo-osmotic perfusion) and inhibition of proteolysis. In the same study, infusion of amino acids in normo-osmotic perfusion of rat liver was also found to inhibit proteolysis. Glutamine and glycine infusions had antiproteolytic effects due to the changes that they elicit in cell volume. The protein-sparing effect of alanine, proline, and serine was 2 x greater than that found when comparable degrees of cell swelling were induced by either hypo-osmotic exposure

or infusion of glycine or glutamine, while phenylalanine had 4x greater antiproteolytic effect than the comparable cell volume increase. In a more recent study, increased cellular water was found to decrease whole-body oxidation and proteolysis (Berneis et al., 1999). Secondary to this finding was an increase the availability and oxidation of free fatty acids for energy, thereby sparing glycogen (Berneis et al., 1999) and, in a cyclical fashion, potentially amino acids from oxidation (see Nair et al., 1988).

The antiproteolytic effect of increased cellular hydration is fairly consistent in the literature, but, with one exception (Millar et al., 1997), increases in cell volume above normal have had little effect on protein synthesis (Stoll et al., 1992; Berneis et al., 1999; Haussinger et al., 1994). It is however noteworthy that some osmotically active substances do increase synthesis of contractile tissue (Ingwall et al., 1974) and enhance protein synthesis after exercise in humans (Biolo et al., 1997; Tipton et al., 1999). With an enlarged amino acid pool, through exogenous (e.g. Cr) and/or endogenous (e.g. decreased oxidation) sources, it has been demonstrated that enhanced post-exercise protein synthesis occurs in man (see Biolo et al., 1997). Additionally, if amino acid transport is increased by cell swelling alone (Low et al., 1997) then an anabolic signal is present with cell swelling, albeit indirectly.

For these reasons, cell swelling has been implicated as another possible way by which Cr may function to increase net protein accretion. Interestingly, any gain in mass observed during a short-duration supplementation study (< 10 d) is likely a result of water retention. Mechanisms involving increased protein synthesis would not likely yield a

measurable increase in lean body mass in the short-term studies. The benchmark for detectable changes to take place in muscle mass and strength gains with training is generally 4 - 6 weeks (Hickson et al., 1994; Staron et al., 1994). In a strength-trained population, this timeline will be extended (Hickson et al, 1994).

These results lend compelling evidence for several possible roles of Cr in muscle protein synthesis. Of importance, some of the aforementioned effects may occur simultaneously, as in the case of cell swelling and enhanced contractile function. Whether or not this is the case, the effect of Cr supplementation on muscle protein synthesis certainly warrants future investigation. The implications for strength training athletes are paramount; the result of enhanced protein synthesis is an increase in muscle mass, leading to enhanced force production during contraction and increased stress on contractile tissue, which in turn leads to an increase in training stimulus (Baechle, 1994).

1.3.3 Safety

There has been recent concern in popular literature regarding the possible negative effects of Cr supplementation. There has been one case report of renal function deterioration with Cr supplementation (Pritchard and Kalra, 1998). This subject was suffering from focal glomerulosclerosis prior to supplementation (15 $g \bullet d^{-1}$ for 7 d, then 2 $g \bullet d^{-1}$ for 7 weeks). The renal deterioration was characterized by a 40% increase in serum creatinine (indicative of decreased creatinine clearance at the kidney), which returned to

normal 1 month after discontinuation of Cr supplementation. Kreider et al., (1998) also showed an increase (23%) in serum creatinine in normal, healthy Cr-supplemented (15.75 $g \bullet d^{-1}$ for 28 d) college football players; however, this observation was not characterized as renal deterioration. Further, in short-term supplementation studies involving normal subjects, these results are not substantiated (Harris et al., 1992; Poortmans and Francaux, 1999). These observations do extend a cautionary note to persons at risk to develop renal dysfunction, and strongly suggest that persons with existing renal disease avoid Cr supplementation.

There has also been publicity regarding the deaths of 3 extremely dehydrated wrestlers who were supplementing with Cr. Although Cr intake was implicated in these deaths, the Centers for Disease Control report on the incident (MMWR, (1997) 47:105-108) stated that Cr was not the cause. Oopick et al. (1998) have suggested that Cr loading may still occur in dehydrated subjects. Even so, it is this author's recommendation that persons who wish to supplement with Cr should observe the consumption instructions on commercially available products, which suggest that a proper hydration state be maintained during the supplementation period.

Additionally, recent experimentation has suggested that prolonged Cr supplementation (4 d of 4x5 $g \bullet d^{-1}$, followed by 20 weeks of 1x5 $g \bullet d^{-1}$) may result in temporary inhibition of endogenous Cr synthesis (VanDenBerghe et al., 1997). This would have implications on several organs dependant on Cr for normal function (e.g.

eyes, testes). However, internal Cr production in the above study was found to return to normal within 4 weeks of supplement cessation (VanDenBerghe et al., 1997).

Further concerns exist with Cr effects on gastro-intestinal tract, heart, nerve, muscle, and liver function, but additional investigation is necessary to clarify the safety of Cr in health and disease. For review, see Juhn and Tarnopolsky (1998b).

1.4 Limitations

Research in the area of Cr supplementation is not conclusive. Claims of performance enhancement during supplementation found in popular literature, though wide spread, are often exaggerated or unfounded. Care needs to be taken to ensure adequate sample size for statistical power. Appropriate dietary control must be observed during supplementation to prevent nutritional co-intervention. Further, little experimentation has been undertaken in the area of gender differences.

1.4.1 Gender

There has been little work performed on the effects of Cr supplementation on females specifically (Thompson et al., 1996; VanDenBerghe et al., 1997) or in studies that included both genders (Burke et al., 1996; Grindstaff et al., 1997; Jacobs et al., 1995; Mujika et al., 1996; Prevost et al., 1997; Redondo et al., 1996; Rossiter et al., 1996).

Indeed, only 2 studies to date have statistically compared the effects of supplementation on both genders (Mihic et al., 2000 (body composition and blood pressure), and MacLennan and Tarnopolsky, 2000 (performance and lactate production)), and both studies took place in this laboratory.

It is known that many female athletes have a lower protein intake than males (Phillips et al., 1993). Given that Cr is endogenously made with three amino acids, one may hypothesize that females would have lower total-muscle Cr levels. According to the findings of Greenhaff et al. (1994), subjects with low initial [TCr] are most likely to "respond" to Cr feeding with the greatest increases in [TCr] post-supplementation; thus, it is possible that females would be more likely to respond to Cr supplementation. Interestingly, experimental evidence has demonstrated that females have marginally higher [TCr] (although not significant) compared to males (Forsberg et al., 1991). If this observation is consistent, females may not respond to Cr loading as readily as males (Harris et al., 1992; Casey et al., 1996). Indeed, experimentation in our laboratory has demonstrated that Cr-supplemented males increase in mass 50% more than do similarly supplemented females (Mihic et al., 2000).

There is also the issue of a fibre-type specific loading effect (e.g., Greenhaff et al., 1994). If creatine exerts its influence primarily on type IIb muscle fibers (Vannas-Sulonen et al., 1985) and it is widely accepted that women have a lower volume density of these fibers (Bell and Jacobs, 1990; Sale et al., 1987), it may be that Cr has more of an ergogenic effect on males. Contrary to Vannas-Sulonen, a more recent training study,

which examined the effect of Cr loading on fiber type-specific changes in cross-sectional area in man (Volek et al., 1999), found no evidence of fiber type-specific hypertrophy. Vannas-Sulonen et al. (1985) used subjects who began experimentation with severely atrophic type IIb fibers, which may help to explain the observed fiber type-specific effect, but there is a possibility of a similar effect in normal humans (Greenhaff et al., 1994). These hypotheses highlight the need to perform research in the area of gender differences.

1.4.2 Diet

In any study examining performance there are certain important factors that must be controlled in order to adequately assess any possible egrogenic effect. In addition to the inclusion of equipment familiarization trials, a homogeneous subject group, and similar time of day of testing, drug and nutrient intake prior to performance trials need to be carefully controlled. This need is highlighted with Cr supplementation research in several recent studies.

Harris et al. (1992) and Greenhaff et al. (1994) demonstrated that subjects who have a lower initial [TCr] are more likely to respond positively (as per Casey et al., 1996) to Cr supplementation. A slightly lower intramuscular [TCr] has been shown in people with a low exogenous Cr intake, such as vegetarians. It would therefore be advisable to exclude vegetarians from the subject pool of a Cr supplementation experiment to avoid bias.

Control over timing of drug and nutritional intake are also important to successful supplementation research. Varying a subject's carbohydrate (Hargreaves et al., 1987; Ivy et al., 1988; Lambert et al., 1991) or caffeine (Graham and Spriet, 1991; Powers and Dodd, 1985) intake may have profound effects on performance. Compounding these effects, carbohydrate ingestion has been shown to enhance skeletal muscle Cr uptake during the supplementation period (Green et al., 1996), but simultaneous consumption of Cr and caffeine has been shown to negate any ergogenic effect of Cr supplementation (VanDenBerghe et al., 1996). It is imperative that any Cr supplementation study enlists strict dietary control.

1.4.3 Previous Study

Recent experimentation in this lab has revealed that Cr supplementation does enhance maximal anaerobic performance variables in men and women (MacLennan and Tarnopolsky, 2000). There were shortcomings of interpretation in this experiment, the first of which was that there were no measurements of intramuscular [TCr] or [PCr]. Without these measures, one can only hypothesize as to the mechanism of the observed increase in performance. Closely related, the second shortcoming is simply that weight was not an outcome measure, and body composition was not measured. Several studies have used an increase in body mass as an indication that Cr supplementation has been

effective. Although these two shortcomings do little to complicate interpretation of the results, the efficacy of our supplementation protocol should be further investigated.

1.5 Methods Used in the Current Study

1.5.1 DEXA (Dual Photon X-Ray Absorptiometry)

Since its inception in the late nineteen-sixties, the clinical use of DEXA has been increasing. It is generally employed to provide low cost bone density measures with low radiation dose exposure to patients, with one scan being equivalent to approximately one tenth of a standard dental x-ray, or approximately one week's worth of background exposure from living in Hamilton. It is also a relatively accurate method of body composition measurement. It is capable of giving measures of fat and fat-free mass, as well as bone mineral content and bone mineral density, and the results of fat and fat-free mass are comparable to those obtained through direct measurements (Makan et al., 1997).

1.5.1.1 Methodology

DPX methodology is based on the premise of particle mechanics. A photon is emitted from a source into body tissues and is either absorbed or scattered. During photoelectric absorption, the photon interacts with an inner valence electron of an atom. The electron is ejected and the photon disappears. This causes an outer-shell electron to

shift valence to the inner shell, which emits an x-ray. As a general rule, in tissue this occurs in atoms of low atomic number (C, O, H, N, Ca, P), and the resultant x-ray emissions are of low energy (Webber, 1993). Compton and coherent scattering are the other fates of the photon. On interaction with the atom, the results of Compton scattering are that the photon changes direction, but loses no energy. With coherent scattering, the photon interacts with a loosely bound free electron, changes direction and loses energy (Webber, 1993).

With the Hologic QDR-4500A, photons are emitted at 140 and 70 KeV. They pass through body tissues and the intensity of incident x-rays is measured by a detector on the other side of the body. By comparing the intensity of emitted x-rays from patients to those emitted from a material of known composition (phantom, with sections equal in density to bone, muscle and fat, obtained from pork bone, water and pork lard respectively), the Hologic software calculates approximate masses of the three compartments (Hologic QDR-4500A Fan Scanner Operators Manual, 1992).

1.5.1.2 Rationale for DEXA

With short-term Cr supplementation, the expected differences in lean (and fat) mass are small. Average observed changes are only about 1-2 kg or less than 4% of total mass (Balsom et al., 1993). As such, a sensitive and highly reproducible method is needed to effectively measure these potential changes. DPX methodology using a

Hologic system has been shown to have a very low coefficient of variation on reproducibility measures of both lean (1.4%) and fat (1.8%) mass (Chilibeck et al., 1994). According to sample size calculations carried out on DPX by Chilibeck et al. (1994) a 3% difference in lean tissue mass (approx. 2.1 kg in a 70 kg subject) would achieve significance ($p = 0.05$, power = 0.9) in a sample size as small as 6 subjects using a repeated measures design.

1.5.2 Cr, PCr and ATP assay

Historically, ^{31}phosphate and ^{1}hydrogen magnetic resonance spectroscopy (^{31}P- and ^{1}H-MRS) have been used to quantify intramuscular [PCr], [ATP] and [Cr] non-invasively (Greenhaff et al., 1993b, Taylor et al., 1983). These techniques are quite accurate (Dunnett et al., 1991) and rapid, with sample-to-sample times (single free induction decay) of 2.5s (Francaux et al., 2000). They are, however, limited in that different resonance must be employed for proton and phosphate analysis. In short, separate measures of [PCr] and free [Cr] must be made. Because these analyses occur separately instead of concurrently, the time lag between analyses has been suggested to compromise the estimation of [TCr] *in vivo* (Dunnett et al., 1991).

In order to accurately determine [TCr], a more invasive technique (e.g. Bergstrom needle biopsy technique) is necessary. By this method a skeletal muscle sample is

obtained and frozen. Later, through biochemical or chromatographic analysis, high-energy phosphate and Cr content of the sample may be determined.

Using the Bergstrom technique, Hultman and colleagues (1967) first developed assays to determine the skeletal muscle content of PCr, Cr and ATP. This method is capable of enzymatic analyses of [PCr] and [Cr] from the same sample, and thus, the same time point. Additionally, Juengling and Kammermeier (1980) proposed a method whereby [PCr] and [Cr] could be assayed simultaneously from a muscle sample using reverse-phase ion-pairing high-performance liquid chromatography (HPLC). Although reducing the confounding factors of separate analysis, both of these methods require expensive equipment and supplies, and analysis is a longer process.

Later Dunnett et al. (1991) used muscle samples to compare HPLC and the separate enzymatic assay of Harris et al. (1974), finding the two measurement techniques to be very similar, with r-values of r=0.99 for both [PCr] and [Cr]. Additionally, Soderlund and Hultman (1986) showed an improvement in the accuracy of enzymatic analysis of high-energy phosphates using a delayed freezing technique. By extending the time between extraction and freezing of the muscle sample to 1 min, muscular concentrations of PCr and Cr were more representative of values obtained from ^{31}P-MRS analysis (Soderlund and Hultman, 1986).

With the knowledge that the enzymatic technique is as accurate as HPLC and a superior method for determining [TCr], our laboratory (Tarnopolsky and Parise, 1999) developed an assay similar in method to Harris et al., (1974). Using the delayed-freezing

technique of Soderlund and Hultman (1986), this method enzymatically analyzes human muscle samples for [PCr], [Cr] and [ATP] read by fluorometer.

1.6 Statement of purpose

With questions regarding gender differences, ergogenic effects and body composition not fully addressed, it is apparent that further research needs to be performed in the area of Cr supplementation. The present investigation was undertaken to explore the effects of Cr supplementation on anaerobic power indices, muscular phosphate concentrations and body composition in men and women.

It was our hypothesis that Cr supplementation would result in; an increase in intramuscular [TCr] and [PCr], increased lean body mass, improved anaerobic power production, and improved fatigue resistance during repeated bouts of short duration, maximum intensity exercise.

We further hypothesized that men would demonstrate a greater increase in weight and LBM, but that initial [PCr] and [Cr] will be the same in men and women. We hypothesized that lactate production during a maximal effort would be increased with supplementation, based on previous research in our laboratory (MacLennan and Tarnopolsky, 2000, Tarnopolsky et al, 1996a). We are also the first group to directly compare intramuscular [PCr] and [Cr] in men and women and correlate those measures

with exercise performance, and hypothesized that performance would increase in relation to increases in [PCr].

1.7 References for Chapter 1

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2 MANUSCRIPT

2.1 Introduction

In males, creatine (Cr) supplementation has been shown to increase the intramuscular total creatine (TCr) pool (Harris et al., 1992). This increase has been implicated in providing a larger temporal energy buffer in the form of creatine phosphate (PCr) during short-term exercise, as well as an increased rate of PCr resynthesis (Greenhaff et al., 1994) following such exercise.

$$[TCr] = [PCr] + [Cr] \qquad (1)$$

Previous experiments have demonstrated that a rise in TCr following Cr supplementation is accompanied by an increase in work performed in short-term exhaustive exercise (Birch et al., 1994; Casey et al., 1996).

Recent literature has suggested that there may be a minimal absolute increase in intramuscular Cr concentration that is necessary for an improvement in anaerobic exercise performance (Greenhaff et al., 1994; Casey et al., 1996). In these experiments, subjects exhibiting an increase in TCr concentrations greater than 20 $mMol \bullet kg^{-1}$ dw demonstrated an improvement in short-term exhaustive exercise (Casey et al., 1996). Several possible reasons for the variability of response to Cr loading have been mentioned. Facilitation of loading has been demonstrated with the ingestion of carbohydrate (Green et al., 1996), and performance of exercise during the supplementation period (Harris et al., 1992). Also, subjects who have a low TCr

concentration prior to supplementation generally exhibit the greatest increases as a result of Cr loading (Greenhaff, 1994).

Several studies have found benefits from Cr supplementation in anaerobic exercise performance (for review, see Juhn and Tarnopolsky, 1998). In fact, recent literature indicates improvements in: performance during isokinetic cycling (Birch et al., 1994; Kreider et al., 1998; Prevost et al., 1997), muscle torque development during exercise (Tarnopolsky et al., 1997; Greenhaff et al., 1993a), running (Harris et al., 1993), weight lifting (VanDenBerghe et al., 1997; Earnest et al., 1995), power development (Volek et al., 1996) and repeated bouts of maximal effort (Kreider et al., 1998; Volek et al., 1997; Greenhaff et al., 1996).

There is however still some question regarding the overall efficacy of supplementation. Observed changes in performance have been highly variable. Some experimenters have discovered no ergogenic effect of Cr (Snow et al., 1998; Odland et al., 1997), others, an ergolytic effect (Balsom et al., 1993) with aerobic or weight-bearing activities.

There has been little work performed on the differential effects of Cr supplementation between genders (MacLennan and Tarnopolsky, 2000; Mihic et al., 2000). It is generally accepted that female athletes have a lower protein intake than males (Phillips et al., 1993). With less exogenous or dietary Cr, it is expected that females likely have lower TCr level, and thus would be more likely to Cr load (Greenhaff et al., 1994; Harris et al., 1992). On the contrary, recent experimental evidence has suggested

that healthy females exhibit a non-significant trend toward higher [TCr] at rest as compared to males (Forsberg et al., 1991), possibly due to greater endogenous Cr synthesis. Despite this, VanDenBerghe et al. (1997) have shown that females do achieve significant benefit from Cr supplementation during weight training.

Recent experimentation in this laboratory has indicated that there may be a gender-difference in the effect of Cr supplementation on lean body mass (LBM). Mihic et al. (2000) found a trend towards increased LBM in both genders. However, in men taking Cr a 2% increase in LBM was observed, while identically supplemented women increased only 1%. If women are able to Cr load to the same extent as men, but there is a difference in the effect of Cr supplementation on performance, LBM or lactate, then there may be a gender-difference in sensitivity to the effects of Cr.

Additionally, experiments have failed to establish an effect of elevated [TCr] on blood lactate concentration following a maximal effort. Some researchers have discovered an increase during Cr loading (Greenhaff et al., 1994; Tarnopolsky et al., 1997), others have found a decrease (Balsom et al., 1993; Prevost et al., 1997) while most have found no change in concentration of lactate when exercise is performed following a supplementation period (Birch et al., 1994; Bosco et al., 1997; Dawson et al., 1995; Greenhaff et al., 1993a; Odland et al., 1997; Snow et al., 1998; Terillion et al., 1997).

There are two demonstrated roles of Cr in glycolytic flux; one states that phosphocreatine inhibits PFK activity, thus reducing the production of lactate; the other asserts that free Cr stimulates PFK (Storey et al., 1974). Although these effects are

representative of typical negative-feedback enzymatic control, varying interpretations of the data have rendered opposite conclusions regarding the effect of Cr on lactate production.

It was our hypothesis that Cr supplementation would result in: an increase in intramuscular [TCr] and [PCr], increased lean body mass, improved anaerobic power production, and improved fatigue resistance during repeated bouts of short duration, maximum intensity exercise.

We further hypothesize that men will demonstrate a greater increase in weight and LBM, but that initial [PCr] and [Cr] will be the same in men and women. We hypothesize that blood lactate concentration following maximal effort will be increased with supplementation, based on previous research in our laboratory (MacLennan and Tarnopolsky, 2000, Tarnopolsky et al, 1996a). We are also the first group to directly compare intramuscular [PCr] and [Cr] in men and women and correlate those measures with exercise performance, and hypothesize that performance will increase in relation to increases in [PCr].

2.1 Methods

2.2.1 Subjects

Twenty-seven healthy, physically active volunteers were recruited to participate in the study (14 female and 13 male). Written informed consent was obtained from each participant, and the McMaster University Ethics Committee approved the study. All volunteers were familiarized with the apparatus and testing procedure prior to data collection. Prior to trial one, subjects attended the lab to receive instruction, and collected a 4-day diet record in order to create a dietary checklist to precede the experimental protocol outlined below, and to ensure that no subject was a vegan vegetarian. Table 2.1 presents the physical characteristics of the subjects.

Table 2.1: Physical Characteristics of Subjects

		Height(cm)	Weight(kg)	Age(y)	body fat(%)
Females(n=14)	Cr (n=7)	166.1 ± 6.2	64.7 ± 8.4	23.6 ± 1.3	19.0 ± 4.4*
	Pl (n=7)	166.4 ± 3.6	60.9 ± 2.6	21.7 ± 0.8	20.7 ± 4.7*
Males (n=13)	Cr (n=6)	182.6 ± 7.7*	84.2 ± 15.0*	22.7 ± 3.9	7.3 ± 2.9
	Pl (n=7)	183.8 ± 10.1*	80.1 ± 7.2*	22.3 ± 2.3	11.9 ± 6.3

*$p < 0.001$ value greater than other gender

Subjects were randomly assigned to a double-blind two factor between- (gender and supplement), single factor within- (trial) subjects design. Participants received no supplement for trial one (pre), and either Cr (6 males, 7 females), 5 g, by mouth, four

times a day for 5 days (loading), followed by 5 g, once a day for 5 days (maintenance), or placebo (7 males, 7 females) (pl = glucose polymer (Polycose, Ross Laboratories, OH)) instead of Cr for trial two (post). The powders were placed in identical containers to be consumed thoroughly mixed with juice or milk in four equal doses, and once mixed, were indistinguishable by consistency or taste.

2.2.2 Protocol

Following an orientation session where subjects were exposed to and familiarized with the entire performance testing, and provided the experimenters with informed consent, each participant was required to complete a 20 day protocol outlined as follows;

Day -2; light activity and dietary checklist

Day -1; no exercise and dietary checklist

Day 0; no exercise and dietary checklist prior to **performance test***, DEXA scan

Day 1; light activity and normal diet

Day 2 and 3; no exercise and flesh-free diet

Day 4; no exercise, pre-packaged diet, 24 h urine collection, and **biopsy***

Day 5-11; normal activity and diet

Day 9-13; supplementation

Day 12; light activity and dietary checklist

Day 13; no exercise and dietary checklist

Day 14; no exercise and dietary checklist prior to **performance test***, DEXA scan

Day 14-18; supplementation maintenance (habitual activity, remainder Day 14)

Day 15; light activity and normal diet

Day 16-17; no exercise and flesh-free diet

Day 18; no exercise, pre-packaged diet, 24 h urine collection, infusion/**biopsy***

Performance test* on day 0 and day 14 consisted of the following;

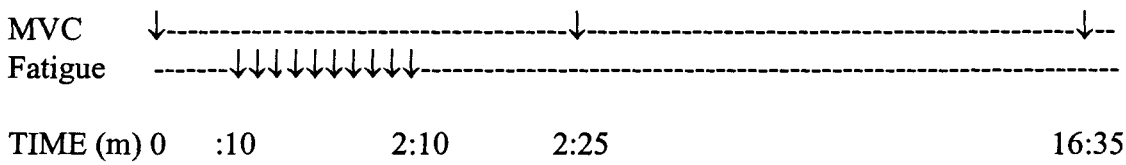
Upon arrival to the lab, the subject's mass was recorded using a pan balance and adherence to diet and exercise checklist was confirmed. A plastic catheter (20 or 22 gauge) was then inserted into the antecubital vein of the subjects' dominant arm. A resting blood sample was taken in a sterile, pre-chilled 5 ml. heparinized, tube and placed on ice until subsequent centrifugation (1500 rpm for 5 min). Subsequent samples were collected in identical fashion. Plasma aliquots were stored at -50°C until analysis for lactate concentration (Model 23L, Yellow Springs Instruments, OH).

Subjects proceeded to a separate room where they performed an ischemic handgrip test as described previously (Tarnopolsky et al., 1997). Subjects were seated with their dominant arm fully extended and parallel to the floor in front of them. They were provided with a handgrip apparatus, consisting of a dynamometer with grip spacing constant at 25 mm. The dynamometer was connected to a force transducer, which continuously recorded force produced during the test. A blood pressure cuff was inflated to 40 mmHg above systolic blood pressure (≈ 160 mmHg) around the upper arm, causing

an occlusion of blood flow to the exercising arm. The subject was then required to maximally squeeze the handgrip apparatus, alternately gripping for 9 s and resting for 1 s. The total duration of the test was 1 min, after which the cuff was deflated. Blood samples were taken at 1 and 5 min of recovery and analyzed as above. Peak force during each of the 6 contractions was recorded.

Following a brief (≈ 10 min) rest period, subjects were then seated with the dominant lower limb secured in a custom-made isometric torque dynamometer as described previously (Tarnopolsky et al., 1997).

Figure 2.1: Fatigue Protocol for the dorsiflexors



Following a maximum voluntary contraction (MVC) torque measurement, a fatigue protocol of the dorsiflexors of the foot commenced, according to procedures outlined by Tarnopolsky et al. (1996), with slight modification (see Fig. 2.1). Briefly, subjects were asked to perform a two-minute intermittent MVC of the muscles responsible for dorsiflexion about the ankle. Contractions lasted fifteen seconds each, and all eight epochs were separated by approximately 2 s of rest. Measurements of torque generation of the dorsiflexors were obtained continuously for the duration of the protocol. At t = +145 s of the testing an MVC was performed to obtain a fatigued torque

measure. A final MVC (at t = +16 min 35 s) was collected to evaluate recovery of torque on completion of the protocol.

Subjects were then removed from the lower leg apparatus and, after a brief rest period (≈ 10 min), were required to sit in a chair equipped with an isokinetic leg dynamometer (Cybex II, Lumex Inc., Rankonkoma, NY). Their right lower leg was secured to the apparatus at the distal tibia, with the knee joint centered at the axis of rotation of the resistance arm, in order to measure torque production of the knee extensors during maximal leg extension. The torso of each participant was secured to the apparatus to minimize co-activation and improve isolation of the quadriceps. Subjects were instructed to contract maximally during extension and relax during the flexion counter-movement. They were provided with a brief warm-up (≈ 1 min) prior to commencement. Knee extension was performed at a speed of $30°•s^{-1}$. Three sets of 10 repetitions were performed at this speed, allowing 2 min. of rest between each set. From previous data, young males and females in our laboratory have a day-to-day coefficient of variation (CV) of ≈ 5% in knee extension. A rest period of 10 minutes followed, leading into a maximal cycling test (Wingate test - Bar-Or, 1987).

Subjects were permitted to perform light stretching during the rest period. Two minutes prior to the Wingate test a 5 ml blood sample (pre Wingate) was taken. Wingate tests were performed on a manually weighted Monarch cycle ergometer (resistance = 7.5% of subject weight ± 100 g), with seat height adjusted to suit each subject and recorded for reference in subsequent trials. Before commencing the test, subjects were

given a countdown 10 s in duration. They were allowed to pedal without resistance until the start of the test. With five seconds remaining in the countdown, subjects were instructed to vigorously increase cadence to reach maximum pedaling rate at zero seconds (the beginning of the test), at which time the weight-loaded brake was instantly applied. Using a sensor which measures the speed of the flywheel on the Monark cycle, measurements of peak and average power, work performed and fatigue were obtained from the number of and change in rate of wheel revolutions per unit time. The duration of each test was 30 s and the final blood sample was drawn at 3 min following the Wingate Test.

Biopsy* on days 4 and 18 occurred as follows:

Resting tissue samples from the *vastus lateralis* muscle were obtained on each occasion using a percutaneous needle biopsy technique (Bergstrom needle) aided by manual suction. Approximately 100 - 150 mg of tissue was extracted under local anesthesia (Xylocane), immediately removed from the needle, trimmed of any visible fat or connective tissue, placed in a micro-centrifuge tube and snap-frozen in liquid nitrogen.

At a later date, biopsy samples were lyophylized, powdered and dissected free of visible connective tissue and blood in a 4°C climate chamber as per methodology described in Tarnopolsky and Parise (1999). Briefly, lyophilized muscle samples were placed in a sterile, dry petrie dish where pieces of connective tissue were removed using a pair of sterile tweezers. Sample fractions were then powdered between the rough surfaces of two pairs of tweezers. Pulverized muscle tissue flaked into the petrie dish during this

procedure while additional connective tissue remained whole; any connective tissue discovered in this manner was also removed. After each muscle tissue sample was removed, the petrie dish was flushed with ethanol and all debris and liquid were removed using a sterile, lint-free gauze (Kim-wipe). Dishes were allowed to dry ≈ 5 min. between each sample powdering. The weight of the powdered and dissected tissue was carefully recorded using an electronic scale, and then stored in dessicant at -50°C in a 1.5 ml polyethylene tube for subsequent analysis of PCr, Cr and ATP (for procedure, see section 2.2.5).

2.2.3 Data Acquisition and Recording

Force produced by the finger flexors during the one-minute handgrip test was obtained using a custom-designed handgrip dynamometer. This was linked to computer driven data acquisition software through a Dataq Waveform scrolling board (WFS-200 DC: Dataq Instruments Inc., Akron, OH) by which force produced was displayed in real time on a VGA computer monitor. Subsequent analysis of stored data was accomplished using CODAS data analysis software (Dataque instruments Inc., Akron, OH). Details of this testing protocol have been described in Tarnopolsky et al. (1997).

Torque measures of the ankle dorsiflexors were performed using a custom-made boot-type apparatus. Methods and apparatus used in recording were similar to those previously described (Tarnopolsky et al., 1996). Briefly, the lower limb was secured into

the dynamometer at a knee angle of 90° from extension and an ankle angle of 10° of plantarflexion. All MVC torque values were displayed in real time on a VGA computer monitor and measured at the peak of voluntary effort. Additionally, the data were recorded to hard disc using a Dataq waveform scrolling board (WFS-200DC; Dataq Instruments Inc., Akron, OH). These stored mechanical data were subsequently analyzed using a custom-designed computer-driven oscilloscope and data analysis software (CODAS, Dataq Instruments Inc., Akron, OH).

Knee extensor torque during isokinetic MVC was measured and recorded on a custom-made Cybex-based apparatus (Cybex II, Lumex Inc., Rankonkoma, NY) linked to a real time chart recorder (Hewlett Packard, 7402A). All analysis of the Cybex torque charts was collected and recorded by the same investigator (DM). Data from the anaerobic cycling test was acquired using software from Sports Medicine Industries (St. Cloud, Minnesota), and was later analyzed and recorded (DM).

2.2.4 DEXA Measurements

On the same day as each performance trial, subjects reported to the nuclear medicine laboratory of McMaster University Medical Centre. They were required to remove all metal objects (i.e. jewelry) and to wear only a hospital gown during scanning. Subjects were positioned on the scanning apparatus in the same fashion for each trial and were required to remain perfectly still for the 3 min required to complete the scan. Care

was taken to ensure that participants did not look directly into the fan beam aperture as it passed over their eyes.

Dexa scanning was performed on a QDR-4500 Fan Beam X-Ray Bone Densitometer (Hologic Inc. Waltham, Ma). Subject positioning is specifically outlined in the Hologic QDR-4500 Scanner Operator's Manual. Briefly, during whole-body scans, participants lay supine within a specified area on the scanning table. The densiometer then made three sequential scans (i.e., the right side, center and left side of the subject). Because the scanning area, specified by markings on the scanning table, was limited (190cm x 70cm), one subject was required to slightly bend his knees, and one other had to keep his hands flat against his sides. Placement of each subject was identical for each trial.

Specific operator-defined sub-regions of the body were determined prior to analysis of the original scans. All scanning and subsequent analysis was conducted by the same operator (DM).

2.2.5 Muscle Metabolite Analysis

Perchloric acid (0.5 M) containing EDTA (1 mM) was used to extract muscular metabolites. A ratio of 800 μL of solution was added to every 10 mg of powder, and the mixture was placed on ice while periodically vortexing for 5 minutes. Five minutes of centrifugation at 7000 rpm followed, and then the sample was neutralized using 2 M

$KHCO_3$ while periodically vortexing for 5 min. The sample was then centrifuged at 7000 rpm for 15 min; the supernatant was extracted, and stored at -50°C in a 1.5 mL polyethylene tube until subsequent metabolite assays. Assays were performed using methods previously described (Tarnopolsky and Parise, 1999).

Briefly, high energy phosphates (ATP and PCr) were assayed in the presence of 50 mM Tris buffer, pH 7.4; 0.5 mM dithiothreitol, 100 μM glucose, 1 mM magnesium chloride, 50 μM $NADP^+$, 25 $U \bullet mg^{-1}$ CK, 350 $U \bullet mL^{-1}$ glucose-6-phosphate dehydrogenase, and 280 $U \bullet ml^{-1}$ hexokinase and ADP. Ten μL of 10% bovine serum albumin was used to stabilize CK and hexokinase. All assays were carried out in 13 x 75 glass screw-top tubes with 1 mL of reagent for every 10 μL of sample. Reactant solutions were vortexed and read using a fluorometer (Shimadzu RF-Mini 150, Japan) with emission wavelength at 460 nm and an excitation wavelength of 360 nm. For ATP measurement, 25 μL of hexokinase was added to the solution, tubes were then vortexed and incubated at room temperature in the dark for 30 min, and read in the fluorometer. For PCr analysis, 20 μL of CK was added to solution, tubes were incubated as above for 60 min., and then read by fluorometry again. All measurements of muscular metabolites are expressed in $mM \bullet kg^{-1}$ dry weight.

For the Cr assay, extracts were placed in 50 mM imidazole buffer, pH 7.4; 0.15 mM NADH, ATP, 1 mM magnesium chloride, 1 mM potassium chloride, 0.5 mM phosphoenolpyruvate, 1250 $U \bullet mL^{-1}$ lactate dehydrogenase, 25 $U \bullet mg^{-1}$ CK, and 2000 $U \bullet mL^{-1}$ pyruvate kinase. Again, CK was stabilized using 10% bovine serum albumin.

The samples were added to reagent, vortexed and incubated as above for 15 min. The addition of 25 µL of CK followed, the samples were vortexed and incubated as before for 30 min, then read again.

2.2.6 Statistical Analysis

All data were analyzed using a three-factor (gender, supplement and time - two factor between-groups (gender and supplement), one factor within-group (time)) or four-factor (additional factor within-group (contraction)) analysis of variance (ANOVA), unless otherwise noted. Data from each gender were then analyzed separately using a two-or three-factor ANOVA respectively. When significant interactions were observed, Tukey (HSD) post hoc analysis was employed to make pair-wise comparisons. Statistical significance was considered to be at a level of $p \leq 0.05$, and all values in text and tables are mean ± standard deviation (SD) based on a sample size of 27 (13 men; Pl, n = 7, Cr, n = 6, and 14 women; Pl and Cr, n = 7) unless stated otherwise. Where power calculations were employed, they were based upon $\alpha=0.05$ and $\beta=0.20$, one-tailed testing using the following equations.

1. Repeated measures design, $N = [(Z_\alpha + Z_\beta) * SD/\Delta]^2$
2. Between groups design, $N/group = 2[(Z_\alpha + Z_\beta) * SD/\Delta]^2$

2.3 Results

2.3.1 Muscle metabolite assay

The Cr loading protocol was successful in eliciting increases in intramuscular [TCr] (refer to Figure 2.2). Both males and females exhibited elevated [TCr] when supplemented with Cr ($p < 0.05$) (refer to Table 2.2). The [PCr] showed a significant interaction (refer to Figure 2.3). Subjects who supplemented with placebo had a small decrease in [PCr], from 72.7 ± 16.8 to 67.4 ± 5.0 mMol•kg^{-1}, while those who supplemented Cr had a small increase (70.9 ± 8.2 to 77.1 ± 15.7 mMol•kg^{-1}; refer to Table 2.3). This interaction was significant at $p < 0.05$, thus post-supplementation, the Cr group had a greater increase in [PCr] versus Pl (≈ 15%). Females showed a trend towards a greater supplementation effect on measures of [PCr]; women increased 11.5 mMol•kg^{-1} to 81.2 mMol•kg^{-1} ($p = 0.11$) while men increased by 1 mMol•kg^{-1} to 73 mMol•kg^{-1} (NSD).

Subjects showed an increase in [Cr] over the course of the study (refer to Table 2.4), from 58.2 ± 10.5 mMol•kg^{-1} at the time of the first biopsy to 67.5 ± 12.5 mMol•kg^{-1} at the time of the second ($p < 0.01$). The Cr-supplemented group had a significantly greater [Cr] throughout the study, with average values of 64.1 ± 12.5 mMol•kg^{-1} as compared to 61.4 ± 12.3 mMol•kg^{-1} in the placebo group ($p < 0.05$).

Table 2.2: [TCr] from muscle biopsy analysis (mMol•kg^{-1} dw).

	Suppl	Pre	Post	Δ
Males	Pl	128.3 ± 15.6	130.8 ± 7.7	2.5
	Cr	125.5 ± 7.9	141.2 ± 12.4*	15.7*
Females	Pl	131.4 ± 13.1	134.7 ± 13.3	3.3
	Cr	134.3 ± 13.6	151.5 ± 25.6*	17.2*

*p < 0.05 Supplement x Time interaction; Cr greater than Pl at Post, NSD between Genders.

Table 2.3: [PCr] from muscle biopsy analysis (mMol•kg^{-1} dw).

	Suppl	Pre	Post	Δ
Males	Pl	74.0 ± 18.4	65.8 ± 3.5	-8.2
	Cr	72.0 ± 6.7	73.0 ± 12.6*	1.0
Females	Pl	71.6 ± 16.7	68.7 ± 5.9	-2.9
	Cr	69.7 ± 9.8	81.2 ± 18.3*	11.5

*p < 0.05 Supplement x Time interaction; Cr greater than Pl at Post, NSD between Genders.

Table 2.4: [Cr] from muscle biopsy analysis (mMol•kg^{-1} dw).

	Suppl	Pre	Post*	Δ
Males	Pl	54.3 ± 12.3	65.1 ± 8.7	10.8
	Cr†	53.5 ± 6.7	68.2 ± 10.1	14.7
Females	Pl	59.7 ± 10.5	66.0 ± 15.6	6.3
	Cr†	64.6 ± 10.3	70.3 ± 15.8	5.7

*p < 0.01 Main effect of Time; Post values greater than Pre. †p < 0.04 Main effect of Supplement; Cr greater than Pl both Pre and Post.

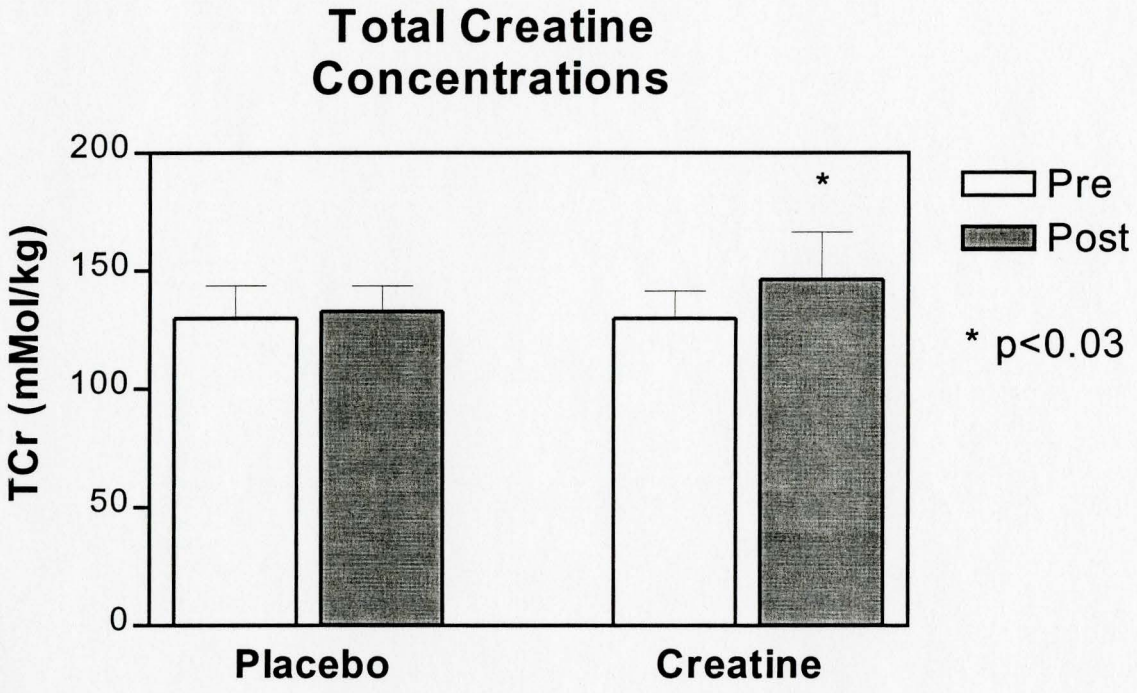


Figure 2.2 Total creatine concentration in $mMol \bullet kg^{-1}$. Creatine group significantly higher post-supplementation at $p < 0.03$.

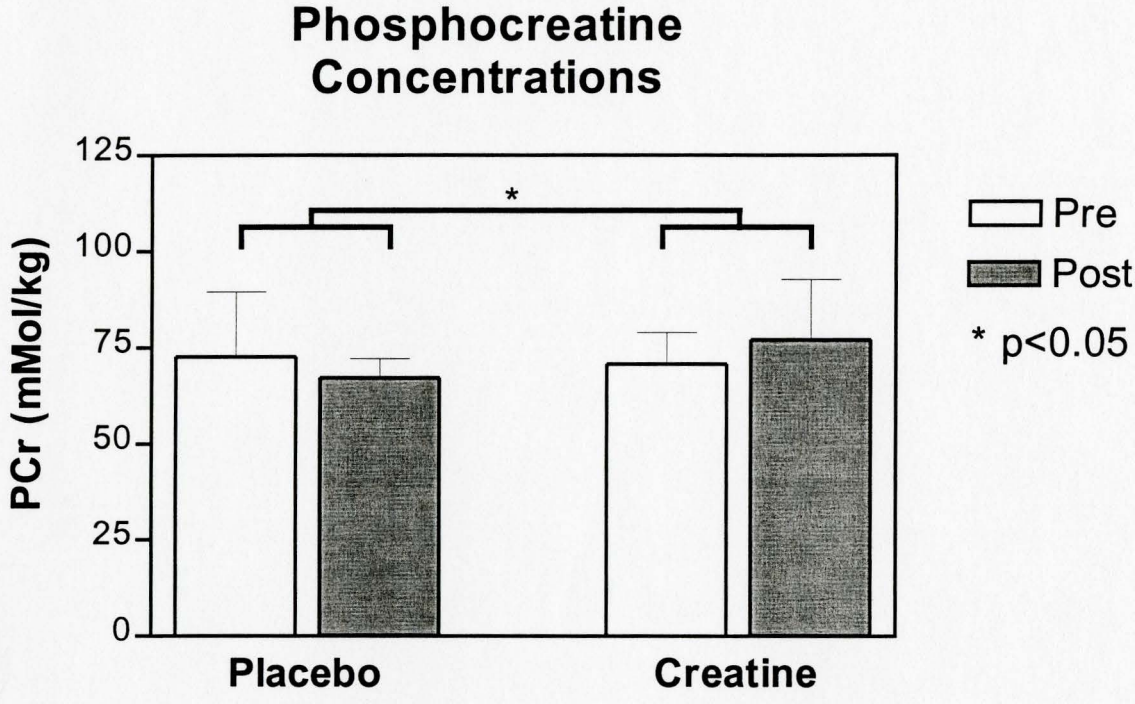


Figure 2.3 Phosphocreatine concentrations ($mMol \bullet kg^{-1}$). Significant interaction such that at Post-test, [PCr] was higher in the Cr group than in Pl ($p < 0.05$).

Measurements of intramuscular [ATP] were also obtained. Values for the placebo group were 21.3 ± 2.9 mMol•kg^{-1} Pre and 22.1 ± 2.2 mMol•kg^{-1} Post, while the Cr supplemented group had values of 21.6 ± 2.1 and 21.3 ± 2.8 mMol•kg^{-1} Pre and Post respectively.

2.3.2 DEXA measurements

Although Cr loading did occur, gains in total mass were not observed. In addition to measures of lean (Table 2.5) and fat mass, bone mineral content (BMC) was also obtained. These measurements were very stable over the two trials, with males having 3050 ± 334 g Pre and 3059 ± 334 g Post, and females having 2300 ± 206 and 2297 ± 210 g Pre and Post, respectively. The correlation coefficient of these measurements across both genders was $r^2 = 0.995$.

Table 2.5: Lean mass of subjects obtained from DEXA measurements (kg).

	supplement	Pre	Post	Δ
Males*	Pl	63.1 ± 3.6	63.8 ± 3.5	0.7
	Cr	66.9 ± 8.1	68.0 ± 9.0	1.1
Females	Pl	46.0 ± 4.2	46.5 ± 4.8	0.5
	Cr	44.6 ± 2.9	45.0 ± 3.8	0.4

*$p < 0.001$ Main effect of Gender; Males greater than Females. No effects of supplementation were observed.

2.3.3 Performance Measurements

2.3.3.1 Wingate Test

In the anaerobic cycling test, Cr-supplemented men showed a significant improvement in peak power and peak power•kg^{-1} body weight compared to men in the Pl group (refer to Table 2.6). Peak power in the Pl group increased by ≈ 2%, while in the Cr group, a ≈ 5% increase was observed. With peak power•kg^{-1} body weight, the increase was greater (pl ≈ 1% vs. Cr ≈ 5%). Cr-supplemented women demonstrated no increase in performance over women in the Pl group (refer to Table 2.7).

As has been previously shown with Wingate testing, males produced significantly more power and performed significantly more work than females ($p < 0.001$).

Table 2.6: Wingate variables for males

	Placebo		Creatine	
	Pre	Post	Pre	Post
Peak (W)	971 ± 72	989 ± 76	994 ± 174	1042 ± 154*
Peak•kg^{-1} (W•kg^{-1})	12.8 ± 1.3	12.9 ± 1.1	11.5 ± 1.1	12.1 ± 1.3*
Ave (W)	690 ± 23	714 ± 38	745 ± 126	749 ± 108
Ave•kg^{-1} (W•kg^{-1})	9.1 ± 0.5	9.3 ± 0.6	8.6 ± 0.9	8.7 ± 1.1
Fatigue Index	49.9 ± 4.9	48.2 ± 3.0	44.9 ± 4.1	48.0 ± 5.5
Total (W)	273 ± 17	279 ± 20	258 ± 27	260 ± 33

*$p < 0.05$ significant Gender x Supplementation x Time interaction; Cr increased more than Pl at Post in males only

Table 2.7: Wingate variables for females

	Placebo		Creatine	
	Pre	Post	Pre	Post
Peak (W)	641 ± 66	666 ± 84	634 ± 72	639 ± 69
Peak•kg^{-1} (W•kg^{-1})	10.3 ± 1.1	10.5 ± 1.3	10.1 ± 0.6	10.2 ± 0.5
Ave (W)	475 ± 48	471 ± 55	437 ± 55	449 ± 51
Ave•kg^{-1} (W•kg^{-1})	7.6 ± 1.0	7.5 ± 1.0	7.0 ± 0.6	7.1 ± 0.5
Fatigue Index	42.1 ± 12.4	46.6 ± 2.7	49.5 ± 4.5	48.0 ± 3.4
Total (W)	229 ± 31	223 ± 30	209 ± 18	214 ± 14

There was no additional difference in Wingate performance as a result of supplementation in either gender. There was, however, a main effect of Time, whereby subjects improved over time on measures of peak and peak•kg^{-1} (from 810 ± 203 W Pre to 834 ± 211 W Post ($p < 0.002$) and from 11.16 ± 1.46 W•kg^{-1} to 11.41 ± 1.52 W•kg^{-1} ($p < 0.02$), respectively). This may be indicative of a need for more orientation trials on the Wingate cycle prior to future investigation.

2.3.3.2 Wingate lactate

Post-exercise lactate concentration was unaffected during the Cr-supplemented trials (Table 2.8). There were gender differences in these measures. Males had significantly more plasma lactate after 30 s of maximal cycling than females (15.0 ± 2.5 mMol•L^{-1} for males as compared to 11.2 ± 3.0 mMol•L^{-1} in females, $p < 0.01$).

Table 2.8: Blood lactate values ($mMol \bullet L^{-1}$) in Wingate test

		Pre		Post	
	supplement	0 min	+3:30 min*	0	+3:30 min*
Males	Pl (n = 5)	1.42 ± 1.68	14.7 ± 2.73[t]	1.88 ± 0.33	16.12 ± 3.01[t]
	Cr (n = 6)	1.68 ± 0.78	13.45 ± 1.59[t]	1.93 ± 0.86	15.78 ± 2.47[t]
Females	Pl (n = 6)	1.62 ± 1.14	11.37 ± 5.58	1.28 ± 0.24	11.12 ± 1.16
	Cr (n = 5)	1.52 ± 0.84	10.90 ± 1.35	1.62 ± 0.61	11.48 ± 2.33

* $p < 0.01$ Main effect of Sample Time; [t] $p < 0.01$ males higher at +3:30 min than females.

2.3.3.3 Dorsiflexor fatigue protocol

Maximum voluntary contractions (MVC) in the tibialis anterior apparatus demonstrated expected gender differences in torque production, although no effect of supplementation was observed (refer to Figure 2.3). Men averaged an output of 47.7 ± 8.3 Nm while women averaged 31.5 ± 6.4 Nm during the first MVC ($p < 0.001$). Although not significant, torque generated both before and after the ten-minute recovery period was higher than that measured immediately after the two-minute fatigue contraction ($p < 0.06$).

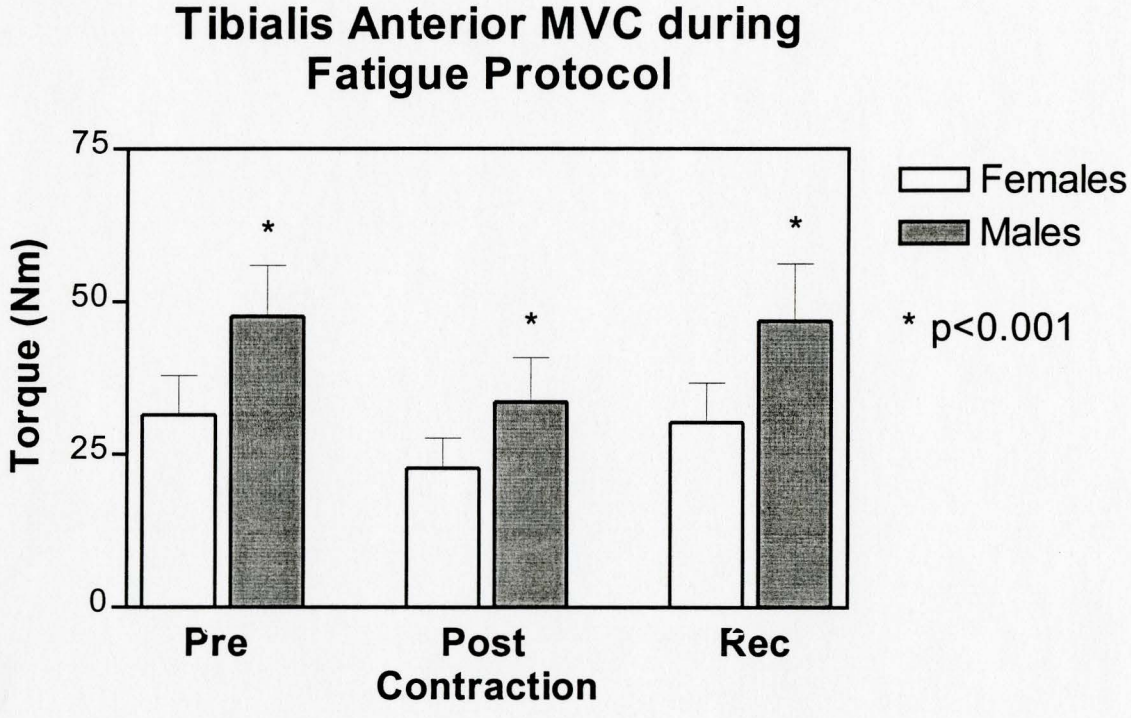


Figure 2.3 Tibialis MVC torque (in Nm) during fatigue protocol. *Males produced significantly more torque than females ($p < 0.001$).

Although not significant, females again tended to be more fatigue resistant than males in absolute terms, falling in torque generation from 31.5 ± 6.4 to 22.8 ± 4.9 Nm (28%) while males dropped from 47.7 ± 8.3 to 33.6 ± 7.3 Nm (30%) ($p = 0.06$ – Figure 2.3). The relative difference in fatigue (brackets) between genders is only 2%. When fatigue is expressed in this manner, there is no significant difference between genders.

2.3.3.4 Cybex knee extension

The predicted differences between genders in torque production were observed. Males proved stronger, producing on average across all six contractions in both trials, 238 ± 62, 250 ± 59 and 256 ± 65 Nm of torque in bouts 1, 2 and 3 respectively, compared to 142 ± 30, 150 ± 29 and 152 ± 31 Nm with females ($p < 0.001$). No differences in fatigue resistance were observed, nor were there any effects of supplementation.

2.3.3.5 Handgrip test

Males produced more force than females (41.6 ± 10.4 N vs. 25.0 ± 5.4 N, $p < 0.001$) and, in absolute terms, females were more fatigue resistant than males (males dropped from a first MVC value of 49.7 ± 8.4 N to a final of 34.6 ± 9.1 N (30%), while females diminished from 29.4 ± 5.4 N to 22.2 ± 4.1 N (24%), $p < 0.001$). Again, when fatigue is expressed in relative terms (brackets) the difference lessens, but remains

significant ($p < 0.05$ using a three-factor ANOVA, gender x delta score interaction). Both groups showed significant torque reduction over the 6 contractions in the ischemic condition ($p < 0.001$). No effect of supplementation was observed (Figure 2.4).

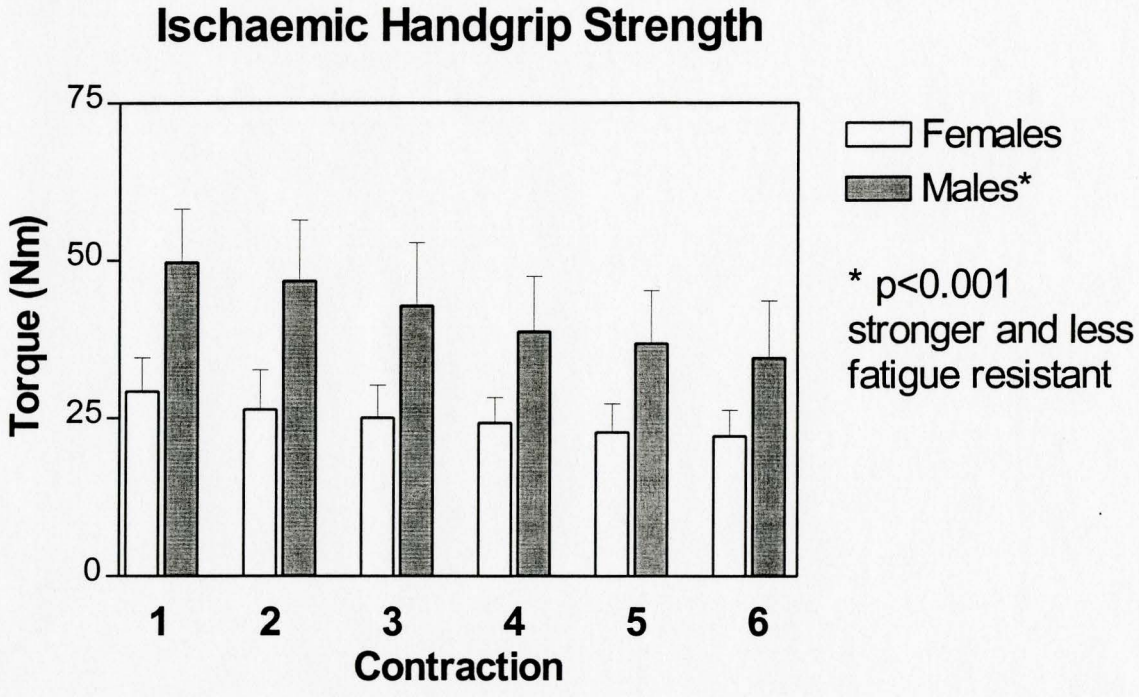


Figure 2.4 Ischaemic handgrip strength measured as force (in N) during MVC. *Males generated significantly greater force ($p < 0.001$) and fatigued significantly more ($p < 0.001$) than females.

2.4 Discussion

2.4.1 PCr, Cr and ATP assay

The results of this experiment support findings that Cr supplementation ($20g \bullet d^{-1} \bullet 5d$) increases intramuscular [TCr] in humans. The average increase in [TCr] ($16.5mMol \bullet kg^{-1}$ dw) is consistent with other experimentation (Greenhaff et al., 1993a, Odland et al., 1997). There was a small but significant increase in the amount of intramuscular [PCr] available at post-test with Cr supplementation, when compared to Pl values, in both genders ($p < 0.05$). The greatest Pre-to-Post difference was seen in females (14.1 $mMol \bullet kg^{-1}$ or 17% compared to males at 9.2 $mMol \bullet kg^{-1}$ or 13%, NSD). There was no statistical difference in the amount of intramuscular [Cr] present pre- or post-supplementation with Cr versus Pl.

It is also noteworthy that men and women are shown to have the same initial level of [TCr]. There has been a considerable amount of controversy in this regard, in light of suggestions by several researchers. Phillips et al. (1993) showed that female athletes have a lower protein intake than their male counterparts. It would follow that these women have less exogenous Cr intake as well (Lykken et al., 1980). If women have lower initial [TCr] as a result, then they may be more susceptible to Cr loading (Greenhaff, 1994). Conversely, Forsberg et al. (1991) suggest that women have a greater initial [TCr] than men, despite no significant difference observed in their results. The current results agree with those of Forsberg et al. (1991), since there were no significant

differences between genders in the pre-supplementation concentration of TCr. Also, dietary analysis in the present experiment (data not printed) revealed that there were no significant gender-differences in the amount of protein consumed•kg^{-1} body weight, which is in agreement with the aforementioned theories.

2.4.2 DEXA analysis

DEXA analysis of body composition showed no effect of Cr on fat or lean body mass. Correlation coefficients were performed on bone mineral content (BMC) Pre- and Post-supplementation data from DEXA analysis in order to provide an estimate of reliability. This measure has been previously used as an index of reliability (Chilibeck et al., 1994), as he found that little variation in BMC would occur over the length of this experiment. As mentioned previously, BMC was highly reproducible. The explained variance was $r^2 = 0.995$, indicating that the DEXA protocol was sufficiently accurate and consistent to yield reliable results.

It may be that increases in [TCr] as a result of Cr supplementation can occur in the absence of significant weight gain. Weight gain in past Cr supplementation research has been thought to be a result of water retention at the cellular level, most likely due to changes in osmotic pressure. If the concentration of a metabolite (e.g. Cr) increases on

one side of a water- (but not metabolite-) permeable membrane that divides a container of water in to two compartments, then water will flow to the compartment containing the metabolite. This type of water retention was implicated in cell swelling, which is hypothesized to enhance protein synthesis (Haussinger et al., 1991), and has led to much speculation that Cr could be an anabolic agent. Assuming that Cr loading occurred without significant gain in lean mass, the hypothesis that water retention may occur in muscle cells as a result of changes in osmotic pressure seems unlikely. It follows that cell swelling would play a negligible role in the current theory regarding Cr as a direct anabolic agent. If an anabolic stimulus is present, it would be apparent over a longer duration of supplementation, most likely coupled with strength training.

One additional hypothesis to explain the lack of weight gain as a result of effective supplementation may be a de-training effect. Over the duration of the 21-day study, only 7 full days of normal activity were permitted. The habitual level of activity in our subject group was greater (men = 3.6 ± 1.2 days of activity•week^{-1}, women = 3.4 ± 1.4). Muscle soreness as a result of biopsy procedures prevented most subjects from resuming normal activity within the 7 days of normal activity. In addition to possible de-training effects, subjects were also required to alter their normal diet (dietary checklist, flesh-free checklist or pre-packaged diet as opposed to food *ad libidum*) for 10 of 21 days. Although all diets were prepared as iso-nitrogenous and iso-energetic compared to their habitual diet, dietary composition may have been affected. These interventions may have altered body mass to render water gains from Cr supplementation undetectable.

Further, fluid consumption during experimentation was not monitored. To be certain of either of the above hypotheses regarding body mass, future experimentation should attempt to control for activity and fluid intake, most easily accomplished with subjects confined to a metabolic ward.

2.4.3 Anaerobic performance

Production of peak power and peak power•kg^{-1} body weight were significantly elevated in Cr-supplemented men vs. Pl, but not women. This gender difference in performance cannot be explained by any change in intramuscular [PCr] (non-significantly greater increase shown in Cr-supplemented women compared to men), lactate (no difference) or lean mass (no difference).

2.4.3.1 MVC performance

No additional performance variables were affected by Cr supplementation. No differences in torque production in the knee extensors were observed with supplementation, nor was a difference evident when the delta score of the performance data was correlated with the change in [PCr] for each subject. One may have expected

differences to be apparent here, as has been demonstrated with fatigue resistance in similar types of contraction (Greenhaff et al., 1993a). With elevated muscular Cr stores through supplementation, and enhanced (or the same) cCK activity, one would expect, provided sufficient recovery, to observe improved fatigue resistance in repeated bouts of exercise. CK rephosphorylates 70% of free Cr in approximately one minute (Tesch et al., 1989). Thus, by definition, a "sufficient recovery" should be no less than one minute. In the present study, recovery was 90 seconds, which should have been adequate time to uncover any possible differences in torque generation. A possible explanation for the current result is variability in measurement: the day-to-day coefficient of variation of measurements in young men and women in this lab on isokinetic knee extension have been shown to be = 5%. While quite reasonable, this degree of variability renders the expected difference in performance as a result of supplementation (3-5%) difficult to detect.

Conversely, the limiting factor may lie in the duration of the contraction and/or the duration of recovery between each contraction. In the present study, activity involving maximal effort of longer than 3-4 seconds showed a slight trend toward improvement in performance with Cr supplementation. This is not surprising in light of existing research. Odland et al. (1994) and also Gitianos et al. (1993) have made observations indicating that PCr supplies the majority of phosphorylation potential in maximal exercise for only 3-5 seconds. With this in mind, it is likely that performance enhancements would be seen in *maintenance* of contractile force, and not necessarily in

the peak production of force. Alternately, peak power may be increased via an increase in SERCA 2 activity (more ATP from PCr) and a corresponding increase in crossbridge cycling, but this hypothesis remains to be tested.

There were also no observed differences with Cr supplementation in the fatigue protocol of the dorsiflexors. Previous studies have suggested that supplementing Cr renders a subject more fatigue resistant in repeated bouts of maximal exercise (Greenhaff et al., 1993a). The present finding is likely a result of insufficient recovery between the fatiguing bout and testing of the fatigued MVC. This protocol involved only one second of recovery for every fifteen seconds of contraction (total duration of two minutes for fatiguing contractions), and only a 15 s recovery before fatigued MVC testing. As such, any effects of Cr or fatigue may not have been apparent.

2.4.4 Lactate

The present experiment uncovered no significant change in post-exercise lactate concentration with Cr supplementation. The reason for this observation may be inadequate sample size. Due to inconsistent catheterization, blood samples could only be obtained for a fraction of subjects (n = 22). Despite a reduced sample size, a 5% increase in lactate production was observed during Wingate testing while supplementing with Cr.

This finding is congruous with observations made previously in this laboratory (MacLennan and Tarnopolsky, 2000). An increase in lactate produced during maximal exercise with Cr supplementation may be related to increases in [PCr].

In 1994, Odland et al. discovered a linear relationship between PCr utilized during maximal exercise and the amount of lactate produced. If there is an increase in [PCr] at rest with supplementation, and this concentration is depleted through maximal exercise (as shown by Harris et al., 1976; Katz et al., 1986; Miller et al., 1987; Yoshida & Watari, 1992), it is possible that there would be an increase in lactate produced during the exercise bout.

Exercise results in the conversion of PCr to free Cr, in order to provide a phosphorylation potential to attach a third phosphate to ADP. It has been suggested that the attendant increase in free [Cr] upregulates glycolysis at the level of phospho-fructo-kinase (Meyer et al., 1986). In anaerobic conditions, or in conditions where energy demand exceeds the rate of ATP production through aerobic means, the enhanced glycolytic flux allegedly caused by free Cr accumulation would lead to the production of lactate. Here again, given that supplementation increases the intra-muscular concentration of PCr which is subsequently depleted to near zero through exercise (McCann et al., 1995), the attendant increase in free [Cr] would lead to an elevation in lactate production. Despite these findings, no correlation was discovered between [PCr] and [lactate].

2.4.5 Gender differences

The present experiment has demonstrated that men and women respond to Cr-supplementation in a similar fashion and direction. [TCr] increased similarly, from a similar starting value in both genders, and lactate production and body composition were unaffected by Cr supplementation. It is noteworthy that females may be more susceptible to increases in [PCr] than men with supplementation, but that men demonstrated slight improvements in peak power and peak power•kg^{-1} body weight generated during Wingate testing, while women did not. These results combined may indicate a potential gender difference in sensitivity to the effects of Cr loading on performance.

One possibility is that females were simply given a higher dosage of Cr relative to body mass than males (0.31 g•kg^{-1} vs. 0.25 g•kg^{-1} respectively), but this does not explain the performance difference. An alternate explanation for a possible gender difference in sensitivity to Cr supplementation involves muscle fiber composition. As previously mentioned, men have a greater volume-density of type IIb muscle fibers, while females have a greater proportion of type I (Bell and Jacobs, 1990; Sale et al., 1987). Cr supplementation may have a greater ergogenic effect on type II fibers, while being transported more easily into type I. Evidence for fiber-type-II-selective effects of Cr exist in beta-guanidinopropionic acid (B-GPA) supplementation research with rats (Ren et al., 1995) and in studies of human gyrate atrophy patients (Vannas-Sulonen et al., 1985), while evidence for type-I-selective loading was shown in Willott et al. (2000).

Feeding of the Cr analogue B-GPA over a period of at least ≈6 weeks has been shown to result in depletion of muscle [Cr] and [PCr] in rats. Ren et al. (1995) discovered a decrease in expression of fast-twitch muscle myosin isoform and an increase in slow twitch isoforms in rats fed B-GPA for 6 weeks. Additionally, Levine et al. (1996) showed a 33% decrease in cross-sectional area of type II(b,x) muscle fibers at the end of 18 weeks of B-GPA feeding in rat diaphragm muscle, while the size of type IIa and I fibers did not change. Gyrate atrophy is a disease that interferes with endogenous Cr synthesis and results in low intramuscular Cr and a progressive atrophy of type II muscle fibers of the skeleton and the eye (see section 1.3.2.1). When subjects with this disease are Cr-supplemented, degeneration of type II skeletal muscle fibers is reversed (Vannas-Sulonen et al., 1985).

Willott et al. (2000) demonstrated that although rat soleus muscle (predominantly type I fibers) had a 34% lower [TCr] than rat extensor *digitorum longus* (predominantly type II fibers) *in vivo*, the rate of Cr uptake at physiologically high concentrations (100 $\mu Mol \bullet L^{-1}$ - see Harris et al., 1992) was 45% greater. The authors suggest that type I fibers may have a lower Km for Cr uptake, likely due to lower initial values of [TCr].

These findings suggest that type IIb muscle fibers are dependent on Cr for maintenance of normal size while type IIa and type I fibers are not, but that type I fibers are able to transport Cr into the cytosol more efficiently at physiological concentrations. It is presently proposed that Cr supplementation, where successful, exerts a greater

supplementation effect on type I fibers, but a greater ergogenic effect on type II fibers; thus it may be more effective in men than in women.

While compelling, the suggestion that there is a gender difference in sensitivity to the effects of Cr supplementation is not wholly substantiated. Previous experimentation in this laboratory has provided dissimilar results. MacLennan and Tarnopolsky (2000) have shown that performance increases in men and women with Cr supplementation are the same.

Males produced more absolute and relative peak and average power than females, possibly because of their greater lean mass or greater volume-density of fast-twitch fibers. Evidence for this is the fact that males had 34% greater blood lactate accumulation during the Wingate test. In general, the data from isokinetic knee extension showed males capable of generating 68% more knee-extension torque than females. With the dorsiflexors, which are much less likely to be strength-trained, again, males produced 50% more torque. Females tended to be more fatigue resistant, but this trend was not significant ($p = 0.06$). Handgrip testing provided similar results as well. Again, males proved capable of producing 69% greater torque than females; however, during the 6 ischemic contractions, males experienced a 30% decline in torque production, while females dropped only 24%. These types of results have been observed previously on numerous occasions (see Fitts, 1994 for review), and it can be concluded with relative certainty that males are generally stronger than females, while females tend to be more

resistant to fatigue in absolute terms (less so when fatigue is expressed as a percentage of their maximum values).

2.4.6 Limitations

One limitation of this experiment is the lack of weight difference observed with supplementation. Studies showing performance increases have often exhibited a concomitant weight gain as a result of supplementation. Present results may have to do with the amount of time between trials or possibly in any training, or de-training, which may have occurred between trials. One distinct possibility is the lack of training stimulus which subjects were exposed to during the period between pre and post measures. It is likely that subjects were unable to train normally during experimentation, either because of imposed restrictions on activity, or because of discomfort resulting from invasive procedures.

The most compelling limitation in the present experiment is restricted statistical power. The differences expected in performance were only 3-5%. The study would need to employ a repeated measures design, or the sample size would need to be increased in order to achieve statistical significance. Based on previous experimentation in this laboratory (MacLennan and Tarnopolsky, 2000), a between-groups study of the present

type would need n=31 in each group to establish significant differences in both genders on measures of Wingate peak power and peak power/kg body weight. Unfortunately, the present design and sample size were necessarily imposed by additional measures not detailed in the text (fractional synthetic rate of actin and myosin heavy chain RNA).

Using the current results, to achieve a significant increase in lactate production following intense contraction, we would need complete data from 28 subjects (14 per group). Ironically, this was the number of subjects initially assigned to the study; however, due to complications in blood collection, only 6 subjects/group were analyzed.

Handgrip data indicate that a sample size of 40 subjects would have been sufficient to establish a significant ($p \leq 0.05$) increase in performance on the first MVC with Cr supplementation (9% increase presently observed). Unexpectedly (based on findings of MacLennan and Tarnopolsky, 2000), data from the dorsiflexor MVC protocol suggest that a sample size $n > 100$ subjects would be necessary to demonstrate a significant increase in performance due to Cr supplementation. The same sample size would be required if significance were to be achieved in DEXA measures of lean mass.

It is apparent from current experimentation that statistical power and sample size should be carefully considered in future Cr supplementation research. Researchers should expect the effects of Cr supplementation on MVC and maximal anaerobic performance, should effects exist, to be in the range of 3-5%. Performance of repeated testing with a cohort of the desired subject group will provide data from which

performance mean and variance estimates on the testing apparatus to be used may be obtained. From this information, future researchers can effectively estimate the sample size necessary to satisfy the power requirements (not rejecting the null hypothesis if it is false) of the testing apparatus. This author suggests that, if using a between-groups design with apparatus similar to those presently described, sample size be no less than 20 subjects per group; if a repeated measures design is employed, no less than 16 subjects should be used.

2.5 Conclusions and recommendations

The present experiment demonstrated that Cr supplementation (20 $g \bullet d^{-1}$ x 5 d) results in an increase in [TCr] in each gender, a difference in [PCr] in each gender, but exerts no effect on [Cr] or body composition measured by DEXA. Additionally, basal [PCr] and [TCr] were observed to be the same in men and women, a result congruous with previous experimentation.

A moderate but significant increase in peak power and peak $power \bullet kg^{-1}$ body weight was observed with Wingate testing of Cr-supplemented men but not women; a result not explained by [TCr], [PCr] or lactate production. No other anaerobic

performance indices were affected by Cr supplementation, nor was lactate produced during anaerobic exercise, in either gender.

Previous experimentation conducted in this laboratory with a more powerful study design (repeated measures) did demonstrate improved performance in anaerobic exercise (peak power, peak power•kg^{-1} body weight, MVC of the dorsi-flexors) in both genders using the same Cr loading protocol. Future investigation should carefully consider sample size, based on the magnitude of expected difference in each gender, and the impact of the experimental protocol on a subject's habitual activity. Control of diet and fluid intake are also essential to clarify the role of Cr as an ergogenic aid.

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3 APPENDICES

3.1 Muscle metabolite extraction procedure

1. Bring freeze-dried muscle samples to room temperature in a dessicated container. Weigh muscle samples into eppendorf tubes or 12x75 glass tubes. Pre cool the centrifuge to 0-4°C. Ensure that PCA and $KHCO_3$ neutralize in a 4:1 ratio.

2. Use the following formula to determine the volume of 0.5 M perchloric acid (with 1 mM EDTA) to be added:

   PCA volume (ul) = f.d. muscle weight (mg) x 80

   e.g., for a 5.0 mg sample, 400 ul PCA would be added. If the concentration of the metabolite to be assayed is very low at rest, multiply the weight of resting muscle samples by 40.

   Note that if muscle samples are very small, the minimum amount of PCA, which should be added, is 150 ul.

3. Do not extract more than 10 samples at a time. Add PCA to muscle samples, and vortex gently or "jiggle" tubes to ensure that all muscle is reached by PCA, while making sure that muscle adherence to test tube wall is minimal. Once PCA is added , **maintain samples on ice for the entire extraction procedure.** Leave samples in PCA for 10 min prior to centrifuging.

4. Centrifuge samples in pre-cooled centrifuge for 5 min @ 7000 – 15000 rpm depending on the centrifuge. Muscle should adhere to walls or bottom of the tube after centrifuging. Transfer the supernatant with Pasteur pipette into a tared, eppendorf or 12x75 tube and weigh PCA extract to the nearest mg. **Remember to maintain samples on ice**. Depending on size of sample, the supernatant may also be transferred into a tared eppendorf tube (with needle hole through top to allow CO_2 to escape upon neutralization).

5. Divide PCA extract mass (mg) by 1.25 in order give the extract volume, then divide this volume by 4 in order to calculate the volume of 2.2 M $KHCO_3$ required for neutralization (the required KHCO3 volume can be obtained directly by dividing the PCA extract mass by 4.1). Add the KHCO3 and vortex well until bubbling stops.

6. Centrifuge samples again in pre-cooled centrifuge for 15 min @ 7000-15000rpm. Transfer the supernatant into labeled 12x75 tube or eppendorf tubes and place on ice (if assay is being run immediately) or store @ -80°C. If samples are extremely small, it may be preferable to leave supernatant in tube following centrifugation and pipette sample directly from tube. Some metabolites are labile and must be run on freshly prepared extracts, while for other metabolites the extracts may be stored frozen at -80°C for subsequent analyses. Prior to analyzing frozen extracts, thaw, vortex and centrifuge for 2-3 min at 7000-15000rpm.

3.2 Muscle ATP-PCr assay

Reference:

Passoneau, J.A., and O.H. Lowry. Enzymatic analysis: A practical guide, Totawa, NJ: Humana Press, 1993, pp. 121-123.

Principle:

$$PCr + ADP \xrightarrow{\textit{Creatine Kinase}} Cr + ATP$$

$$ATP + Gluocse \xrightarrow{\textit{Hexokinase}} ADP + G\text{-}6\text{-}P$$

$$G\text{-}6\text{-}P + NADP^{+} \xrightarrow{\textit{G-6-P dehydrogenase}} Gluconolactone + NADPH$$

Reagents:

1. Tris, pH 8.1 (1 M stock solution); fridge
2. Magnesium Chloride (1 M stock solution); freezer
3. Dithiotretol (0.5 M stock solution); freezer
4. Glucose (100 mM stock solution); freezer
5. AP5A (10 mM stock solution); freezer
6. ADP, monopotassium salt (MW = 501.3); Boehr. 236 675
   (preferred to Sigma disodium salt form, which produces much higher blank)
7. NADP (MW = 757.4); Boehr. 128 058
8. Glucose-6-phosphate dehydrogenase; from yeast, grade I
   Boehr. 127 035 (1mg in 1ml; ~ 350 U/mg)
9. Hexokinase (from yeast); Boehr. 1 426 362 (1 ml; ~1500 U/ml)
10. Creatine kinase (rabbit muscle); Boehr. 127 566 (100 mg; ~350 U/mg)
11. ATP, disodium salt (MW = 555.1); Sigma A-7699 (Sigma Ultra) or A-2383 (Grade I)
12. Phosphocreatine (MW = 255.1); Sigma P-7936 or P-6915 (same product; prepared differently)

Buffer:

	50 ml	100 ml	150 ml	200 ml
Tris, pH 8.1	2.5 ml	5.0 ml	7.5 ml	10.0 ml
$MgCl_2$	50 ul	100 ul	150 ul	200 ul
DTT	50 ul	100 ul	150 ul	200 ul
Glucose	50 ul	100 ul	150 ul	200 ul
AP5A	50 ul	100 ul	150 ul	200 ul
NADP (7.9 mg/ml)	250 ul	500 ul	750 ul	1.0 ml

- pH to 8.1 with 1N NaOH, bring to volume and then add G-6-P-DH

G-6-P-DH	5 ul	10 ul	15 ul	20 ul

Enzyme:

1. Hexokinase – Dilute 10 ul Hexokinase in 1000 ul assay buffer.
2. Creatine Kinase – add 2 mg of CK, 2 mg ADP, and 10 ul of 10% BSA to 1 ml of assay buffer (only enough for ~50 samples, since 20 ul used per cuvette)

Standards:

ATP Standards: Stock: 2 mM ATP; dissolve 5.55 mg in 5 ml dH_2O; prepare just prior to use and store on ice (only stable for a few hours).

Std. #	Conc. (mM)	Stock (ul)	dH_20 (ul)
1.	0.15	75	925
2.	0.18	90	910
3.	0.21	105	895
4.	0.24	120	880
5.	0.27	135	865
6.	0.30	150	850
7.	0.33	165	835
8.	0.36	180	820

PCr Standards: Stock: 10 mM PCr; dissolve 127.55 mg in 50 ml dH_2O; store frozen (-86°C) in aliquots (good for 1-2 months).

Std. #	Conc. (mM)	Stock (ul)	dH_2O (ul)
1.	0.1	10	990
2.	0.2	20	980
3.	0.3	30	970
4.	0.4	40	960
5.	0.5	50	950

6.	0.6	60	940
7.	0.7	70	930
8.	0.8	80	920
9.	0.9	90	910
10.	1.0	100	900
11.	1.1	110	890
12.	1.2	120	880

** Note: Depending on study design you will not be running all standards, e.g. if all samples are resting samples you do not have to run std. # 1,2,11,12 etc.

Assay Mixture:

Tris:	50 mM	$MgCl_2$:	1 mM
DTT:	0.5 mM	Glucose:	100 uM
AP5A:	10 uM	NADP:	50 uM
ADP:	100 uM	G-6-P-DH	0.02 U/ml
Hexokinase:	0.14 U/ml	CK:	0.9 U/ml
ATP:	~1-4 uM	PCr:	1-12 uM

Procedure:

Fluorometer

1. Prepare buffer and pipette 1 ml into each cuvette.
2. Add 10 ul dH_2O (blank), standard or extract (in duplicate).
3. Mix, wait 5 min (in dark), then take first reading.
4. Add 10 ul hexokinase, mix, incubate 30 min at room temperature in dark, and take second reading.
5. Add 20 ul creatine kinase, mix, incubate 60 min at room temperature in dark, and take third reading.

Run regression with standards. Formulate a regression equation and substitute delta – blank for value.

3.3 Muscle Cr assay

Reference:

Passoneau, J.A., and O.H. Lowry. Enzymatic Analysis: A Practical Guide, Totawa, NJ: Humana Press, 1993, pp. 121-123.

Principle:

$$\text{Creatine} + \text{ATP} \xrightarrow[\text{Creatine Kinase}]{} \text{P-Creatine} + \text{ADP}$$

$$\text{ADP} + \text{P-Pyruvate} \xrightarrow[\text{Pyruvate Kinase}]{} \text{ATP} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{Lactate Dehydrogenase}]{} \text{Lactate} + NAD^+$$

Reagents:

1. Imidazole (1M stock solution)
2. $MgCl_2$ (1M stock solution)
3. KCl (1M stock solution)
4. Phosphoenol Pyruvate (PEP) (10mM)
5. ATP (Solid)
6. NADH (15mM stock solution)
7. Lactate Dehydrogenase (LDH) (1250 U/ml)
8. Pyruvate Kinase (2000 U/ml)
9. Creatine Kinase (25 U/mg)

Buffer:

	25 ml	50 ml	100 ml
1. Imidazole	1.25 ml	2.5 ml	5.0 ml
2. $MgCl_2$	125 ul	250 ul	500 ul
3. KCl	0.75 ml	1.5 ml	3.0 ml
4. PEP	60 ul	120 ul	240 ul
5. ATP	3.0 mg	6.0 mg	9.0 mg
6. NADH	75 ul	150 ul	300 ul
7. LDH	5.0 ul	10.0 ul	20.0 ul

8. PK	10.0 ul	20.0 ul	40.0 ul
9. CK	********SEE BELOW**********		

Add reagents 1-6, bring to volume with ddH_2O and adjust to pH 7.5. Add reagents 7 and 8.

Enzyme:

1. Add 5 mg of CK to 1 ml of reagent. Add 10 ul of 10% BSA. Mix by inversion.

Standards:

Cr Standards: Stock: 10 mM Cr; dissolve 65.5 mg in 50 ml ddH_2O.

Std. #	Conc. (mM)	Stock (ul)	dH_2O (ul)
1.	0.20	20	980
2.	0.30	30	970
3.	0.40	40	960
4.	0.50	50	950
5.	0.60	60	940
6.	0.70	70	930
7.	0.80	80	920
8.	0.90	90	910
9.	1.0	100	900
10.	1.1	110	890

Concentrations in Assay Mixture:

Imidazole:	50 mM	NADH:	45uM
$MgCl_2$:	5.0 mM	LDH:	0.24 U/ml
KCl:	30 mM	PK:	0.75 U/ml
PEP:	25 uM	CK:	3.6 U/ml
ATP:	200 uM	Cr:	~1-11 uM

Procedure:

1. Prepare buffer and pipette 1 ml into each cuvette.
2. Add 10 ul dH_2O (blank), standard or extract (samples are run in duplicate).
3. Mix, let sit 15 min at room temperature in the dark, then take first reading.
4. Add 25 ul Creatine Kinase, mix, wait 30 min for Cr to react, then take second reading.

3.4 Consent and information form

CREATINE SUPPLEMENTATION: EFFECTS ON MUSCLE PROTEIN SYNTHESIS AND MUSCULAR PERFORMANCE

INFORMATION AND CONSENT FORM

INVESTIGATORS:	DEPARTMENT:	CONTACT:
Dr. Mark Tarnopolsky	Medicine and Kinesiology	x24465
Mr. Sasa Mihic	Kinesiology	x27037
Mr. Dan MacLennan	Kinesiology	x27037
Mr. Gianni Parise	Kinesiology	x27037

PURPOSE:

Oral creatine supplementation has been a popular practice among athletes involved in anaerobic, short-duration, high-intensity activities (e.g. jumping, sprinting, weight-lifting etc.). It has been suggested that oral ingestion of creatine monohydrate of 20g per day for 5-6 days may benefit physical performance by enhancing volitional strength and anaerobic power. It has also been shown that this protocol is usually accompanied by an increase in body mass, and that males tend to gain significantly more weight than females. These changes in mass are likely due to greater water retention by the muscles, which suggests that lean tissue (fat-free mass) is affected. Indeed, a recent work in our lab (Mihic and colleagues, unpublished data, 1997) has demonstrated that the increase in whole body weight was primarily due to increased lean body mass. Body fat was not affected by the supplementation. Therefore, this study will try to address the following questions:

1. Does creatine supplementation have an effect on muscle protein synthesis?
2. Will creatine supplementation benefit muscle performance in short-term, high-intensity activities?

OUTLINE:

You will be one of the 28 male and female volunteers participating in the study. After having recorded your 4-day dietary intake, you will first be familiarized with the performance testing. You will also be asked to abstain from exercise on specific days during the trial.

Following an overnight fast, you will report to the Human Performance Laboratory in IWC for the performance protocol (PPRE). You will undergo the following tests: a) 60- sec. handgrip strength test b) a 2-min strength test by the m.tibialis anterior (front of your shin - lifts your toes off the ground) c) knee extension fatigue protocol (Cybex apparatus), and d) two consecutive 30 sec. all-out bike sprints (Wingate), interspersed with a 4 min. recovery. Prior to and following exercise, small amounts of blood will be sampled (~10ml) through a plastic catheter. The catheter will have been inserted into the antecubital vein of your dominant arm by a trained, certified lab member.

After a three-day rest, you will undergo a stable isotope infusion protocol (ISOPRE). Prior to and on the day of infusion, it will be required that you eat pre-packaged food designed to mach your habitual intake. A prime (1mg/kg body mass) dose of sterile amino acid (leucine) tracer will then be infused via a plastic catheter (see above), followed by a constant infusion (1mg/kg/hr) for the next several hours. Another catheter will be inserted into the other arm for blood collection at different time-points (total amount ~120ml). ~90 min. after the onset of the infusion, Dr. Tarnopolsky will perform a muscle biopsy on the outer portion of your leg (m. vastus lateralis). As well, expired gas will be collected a number of times. The infusion protocol will be completed following another muscle biopsy of the contralateral m.vastus lateralis. A 24hr urine collection will again take place on the day of testing.

On the following day, you will have your body composition (fat-free mass, body fat %, bone mineral density) assessed by DEXA (x-ray scan). At this point you will be supplemented with either creatine (CR) or placebo (PL; sugar-like substance), as assigned in a randomized, double-blind fashion. The substance will be ingested 4 x 5g/d x 5d, and 5g/d for the subsequent 5 days (total of 10 days). To control for dietary creatine intake, the consumption of food will be pre-determined (check-list diet) for the duration of the supplementation.

On Day 6 of the protocol, you will again perform a performance test, identical to that previously described. You will undergo another DEXA scan on Day 9. On Day 10, supplementation will cease, and a leucine infusion will take place in the same way as ISOPRE (see above). Like for the ISOPRE, pre-packaged diet will be consumed, and 24hr urinary sample collected.

DETAILS OF THE PROCEDURES AND POSSIBLE RISKS:

1. Blood sampling. There may be slight bruising at the site of insertion of the plastic venous catheter. The catheter itself is designed to allow for blood to be drawn safely with minimal discomfort to you. It will be inserted by a trained physician or by a trained and certified member of the lab. The total amount of blood taken will be up to 120ml per testing (1/3 cup).
2. Needle biopsy procedure. Involves the local injection of an anesthetic ("freezing") into the skin of the outer thigh area, after which a small (4mm) incision will be made and a small (50-100mg) piece of muscle will be removed using a sterile hollow needle. After the procedure, a suture will be used to close the skin, and ice and pressure will be applied to minimize bruising. The procedure will be performed by Dr. Tarnopolsky, who has done it more than 7,000 times. Complications with the procedure are rare. However, in our experience with athletes, 4/7,000 experience a local skin infection, 6/7,000 have a small lump at the site of biopsy (all disappear with massage after ~1 week), 1/400 have temporary (up to 4 months) localized loss of sensation in the skin at the site of incision, and a few subjects have mild bruising around the incision for 4-5 days. In theory, one could damage a small motor branch of the m. vastus lateralis, which should not affect function (knee extension). Nevertheless, this has not been seen in any of the patients/subjects byopsied by Dr. Tarnopolsky.
3. Stable isotope infusion. ^{13}C leucine has been widely used to examine whole body protein metabolism, as well as muscle protein synthetic rates. The isotope is stable (i.e. non-radioactive), with the natural abundance of ~1.11% of the total body carbon pool. The slight increase in your isotopic enrichment will return to baseline after 24hr. The infusion solution is guaranteed-sterile by the manufacturer.

4. Creatine monohydrate supplementation. Administration of creatine similar to that of this study's has been used in a number of experiments (Harris et al., 1992; Hultman et al., 1996). No side-effects have been observed, except for a transient increase in total body mass (~1kg). This weight gain is mostly due to water retention by the lean tissue, and will return to baseline shortly after the cessation of the supplementation. Anecdotal reports of increased blood pressure and altered kidney function have not been supported by a recent well-controlled, randomized, double-blind trial done by our lab (Mihic et al., unpublished data, 1997). However, it is not known what impact creatine ingestion may have in conditions of dramatic changes in body mass, typical for some combat sports (wrestling, judo, boxing etc.). The press recently reported deaths of two elite wrestlers who had been losing weight in an extremely short period of time. The autopsies revealed severe cases of dehydration and it was stated that both athletes were taking creatine as they were losing weight. This report suggests that caution should be exercised when major weight reduction programs are combined with creatine supplementation.

BENEFITS:

You should be aware that the results of this study will be made available to the scientific community, although neither your name nor any reference to you will be used in compiling or publishing these results. You may withdraw from the study at any time without any adverse repercussions, even after signing this form.

You will receive an honorarium of $195 upon the completion of the study to compensate for your time commitment. Additionally, you will have access to your own data (body composition, average dietary intake etc.), as well as the group data when it becomes available.

You will be able to contact student investigators at 525-9140 (x27037) and/or Dr. Mark Tarnopolsky at any time regarding your questions or concerns about the study. Dr. Tarnopolsky can be contacted at: W- 521-2100 (x6593, or x6443 pager: 2888); H- 527-1295.

I,_____, HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT, AND AGREE TO PARTICIPATE AS A SUBJECT.

_____ _____

SIGNATURE DATE

_____ _____

WITNESS DATE

3.5 ANOVA summary tables

Intramuscular [TCr]. Key; Gender = 1, Supplementation = 2, Time = 3

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	572	1	331	23	1.73	0.20
2	628	1	331	23	1.90	0.18
3	1263	1	103	23	12.29	0.00*
12	123	1	331	23	0.37	0.55
13	5	1	103	23	0.05	0.83
23	615	1	103	23	5.99	0.02*
123	0	1	103	23	0.00	0.95

Tukey HSD for [TCr], 2 X 3 interaction

suppl.	trial	(1) 129.8	(2) 132.8	(3) 129.9	(4) 146.4
1	1		0.88	0.99	0.00
1	2	0.88		0.88	0.01
2	1	0.99	0.88		0.00
2	2	0.00	0.01	0.00	

Intramuscular [TCr], men only

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
2	94	1	153	11	0.61	0.44
3	533	1	104	11	5.13	0.04*
23	282	1	104	11	2.71	0.13

Intramuscular [TCr], women only

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
2	680	1	494	12	1.38	0.26
3	743	1	102	12	7.31	0.02*
23	336	1	102	12	3.31	0.09

Intramuscular [PCr].

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	35	1	214	23	0.16	0.69
2	212	1	214	23	0.99	0.33
3	2	1	109	23	0.01	0.91
12	23	1	214	23	0.11	0.75
13	210	1	109	23	1.92	0.18
23	469	1	109	23	4.30	0.05*
123	22	1	109	23	0.20	0.66

Tukey HSD for [PCr], 2 X 3 interaction, No Significant Interactions

suppl.	trial	(1) 72.8	(2) 67.2	(3) 70.9	(4) 77.1
1	1		0.54	0.96	0.71
1	2	0.54		0.80	0.09
2	1	0.96	0.80		0.41
2	2	0.71	0.09	0.41	

Intramuscular [PCr], men only.

Effect	MS Effect	DF Effect	MS Error	DF Error	F- Value	p-level
2	46	1	148	11	0.31	0.59
3	84	1	122	11	0.69	0.42
23	138	1	122	11	1.13	0.31

Intramuscular [PCr], Women only

Effect	MS Effect	DF Effect	MS Error	DF Error	F- Value	p-level
2	195	1	257	12	0.71	0.42
3	129	1	97	12	1.33	0.27
23	362	1	97	12	3.74	0.08

Intramuscular [Cr]

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	312	1	129	23	2.42	0.13
2	649	1	129	23	5.03	0.03*
3	1093	1	121	23	9.02	0.01*
12	140	1	129	23	1.08	0.31
13	200	1	121	23	1.65	0.21
23	5	1	121	23	0.04	0.84
123	3	1	121	23	0.02	0.88

Intramuscular [ATP]

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	8	1	6	23	1.29	0.27
2	0	1	6	23	0.03	0.87
3	0	1	7	23	0.03	0.85
12	8	1	6	23	1.34	0.26
13	2	1	7	23	0.25	0.62
23	0	1	7	23	0.01	0.94
123	0	1	7	23	0.01	0.93

Total Body Mass

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	3668	1	149	23	24.57	0.00*
2	241	1	149	23	1.62	0.22
3	2	1	1	23	3.61	0.07
12	315	1	149	23	2.11	0.16
13	1	1	1	23	1.97	0.17
23	0	1	1	23	0.01	0.90
123	1	1	1	23	1.03	0.32

Lean Body Mass

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	5333	1	59	23	89.96	0.00*
2	22	1	59	23	0.37	0.55
3	6	1	1	23	6.00	0.02*
12	101	1	59	23	1.70	0.21
13	1	1	1	23	0.57	0.46
23	0	1	1	23	0.10	0.76
123	0	1	1	23	0.15	0.70

Fat Mass

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	1337	1	41	23	32.35	0.00*
2	135	1	41	23	3.27	0.08
3	2	1	2	23	0.95	0.34
12	38	1	41	23	0.91	0.35
13	1	1	2	23	0.51	0.48
23	0	1	2	23	0.01	0.93
123	1	1	2	23	0.40	0.54

Bone Mineral Content

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	7533144	1	153251	23	49.16	0.00*
2	83223	1	153251	23	0.54	0.47
3	34	1	392	23	0.09	0.77
12	191621	1	153251	23	1.25	0.28
13	338	1	392	23	0.86	0.36
23	206	1	392	23	0.52	0.48
123	5563	1	392	23	14.21	0.00*

Tukey HSD for BMC, 1 X 2 X 3 interaction

sex	suppl.	trial	(1) 2313	(2) 2326	(3) 2288	(4) 2268	(5) 2957	(6) 2940	(7) 3131	(8) 3162
1	1	1		0.91	0.34	0.01	0.00	0.00	0.00	0.00
1	1	2	0.91		0.03	0.00	0.00	0.00	0.00	0.00
1	2	1	0.33	0.03		0.58	0.00	0.00	0.00	0.00
1	2	2	0.01	0.00	0.58		0.00	0.00	0.00	0.00
2	1	1	0.00	0.00	0.00	0.00		0.78	0.00	0.00
2	1	2	0.00	0.00	0.00	0.00	0.78		0.00	0.00
2	2	1	0.00	0.00	0.00	0.00	0.00	0.00		0.11
2	2	2	0.00	0.00	0.00	0.00	0.00	0.00	0.11	

Pearson Product-Moment Correlation (Casewise MD deletion, N=27) for Pre-Post BMC

	BMC Pre	BMC Post
BMC Pre	1.000000	.997437
BMC Post	.997437	1.000000

Wingate Test

Effect	Wilks' Lambda	Rao's R	DF 1	DF 2	p-level
1	0.16	15.52	6	18	0.00*
2	0.78	0.82	6	18	0.57
3	0.37	5.07	6	18	0.00*
12	0.74	1.04	6	18	0.43
13	0.76	0.93	6	18	0.50
23	0.50	2.99	6	18	0.03*
123	0.52	2.79	6	18	0.04*

Tukey HSD, 2 X 3 interaction, Peak Power

suppl.	trial	(1) 806	(2) 827	(3) 815	(4) 841
1	1		0.13	0.79	0.01
1	2	0.13		0.51	0.49
2	1	0.79	0.51		0.04
2	2	0.01	0.49	0.04	

Tukey HSD, 2 X 3 interaction, Peak Power/kg

suppl.	trial	(1) 11.53	(2) 11.72	(3) 10.79	(4) 11.11
1	1		0.51	0.00	0.01
1	2	0.51		0.00	0.00
2	1	0.00	0.00		0.08
2	2	0.01	0.00	0.08	

Tukey HSD, 2 X 3 interaction, Average Power - No Significant Interactions

Tukey HSD, 2 X 3 interaction, Average Power/kg

suppl.	trial	(1) 8.36	(2) 8.41	(3) 7.80	(4) 7.90
1	1		0.99	0.01	0.05
1	2	0.99		0.01	0.03
2	1	0.01	0.01		0.93
2	2	0.05	0.03	0.93	

Tukey HSD, 2 X 3 interaction, Fatigue Index - No Significant Interactions

Tukey HSD, 2 X 3 interaction, Total Work

suppl.	trial	(1) 251	(2) 252	(3) 234	(4) 237
1	1		0.99	0.01	0.05
1	2	0.99		0.01	0.04
2	1	0.01	0.01		0.90
2	2	0.05	0.04	0.90	

Tukey HSD, 1 X 2 X 3 interaction, Peak Power

sex	suppl.	trial	(1) 641	(2) 666	(3) 634	(4) 639	(5) 971	(6) 989	(7) 995	(8) 1042
1	1	1		0.57	0.99	1.00	0.00	0.00	0.00	0.00
1	1	2	0.57		0.27	0.46	0.00	0.00	0.00	0.00
1	2	1	0.99	0.27		0.99	0.00	0.00	0.00	0.00
1	2	2	1.00	0.46	0.99		0.00	0.00	0.00	0.00
2	1	1	0.00	0.00	0.00	0.00		0.87	0.62	0.00
2	1	2	0.00	0.00	0.00	0.00	0.87		0.99	0.01
2	2	1	0.00	0.00	0.00	0.00	0.62	0.99		0.02
2	2	2	0.00	0.00	0.00	0.00	0.00	0.01	0.02	

Tukey HSD, 1 X 2 X 3 interaction, Peak Power/kg

sex	suppl.	trial	(1) 10.29	(2) 10.50	(3) 10.11	(4) 10.15	(5) 12.78	(6) 12.93	(7) 11.47	(8) 12.06
1	1	1		0.92	0.97	0.99	0.00	0.00	0.00	0.00
1	1	2	0.92		0.40	0.54	0.00	0.00	0.00	0.00
1	2	1	0.97	0.40		0.99	0.00	0.00	0.00	0.00
1	2	2	0.99	0.54	0.99		0.00	0.00	0.00	0.00
2	1	1	0.00	0.00	0.00	0.00		0.99	0.62	0.01
2	1	2	0.00	0.00	0.00	0.00	0.99		0.00	0.00
2	2	1	0.00	0.00	0.00	0.00	0.00	0.00		0.05
2	2	2	0.00	0.00	0.00	0.00	0.01	0.00	0.05	

Tukey HSD, 1 X 2 X 3 interaction, Average Power

sex	suppl.	trial	(1) 475	(2) 471	(3) 437	(4) 449	(5) 690	(6) 714	(7) 745	(8) 750
1	1	1		0.99	0.16	0.58	0.00	0.00	0.00	0.00
1	1	2	0.99		0.28	0.77	0.00	0.00	0.00	0.00
1	2	1	0.16	0.28		0.99	0.00	0.00	0.00	0.00
1	2	2	0.58	0.77	0.99		0.00	0.00	0.00	0.00
2	1	1	0.00	0.00	0.00	0.00		0.75	0.02	0.00
2	1	2	0.00	0.00	0.00	0.00	0.75		0.40	0.26
2	2	1	0.00	0.00	0.00	0.00	0.02	0.40		0.99
2	2	2	0.00	0.00	0.00	0.00	0.00	0.26	0.99	

Tukey HSD, 1 X 2 X 3 interaction, Average Power/kg

sex	suppl.	trial	(1) 7.63	(2) 7.47	(3) 6.99	(4) 7.13	(5) 9.08	(6) 9.35	(7) 8.61	(8) 8.67
1	1	1		0.99	0.15	0.41	0.00	0.00	0.00	0.00
1	1	2	0.99		0.44	0.81	0.00	0.00	0.00	0.00
1	2	1	0.15	0.44		0.99	0.00	0.00	0.00	0.00
1	2	2	0.41	0.81	0.99		0.00	0.00	0.00	0.00
2	1	1	0.00	0.00	0.00	0.00		0.96	0.53	0.68
2	1	2	0.00	0.00	0.00	0.00	0.96		0.09	0.14
2	2	1	0.00	0.00	0.00	0.00	0.53	0.09		0.99
2	2	2	0.00	0.00	0.00	0.00	0.68	0.14	0.99	

Tukey HSD, 1 X 2 X 3 interaction, Fatigue Index - No Significant Interactions

Tukey HSD, 1 X 2 X 3 interaction, Total Work

sex	suppl.	trial	(1) 229	(2) 223	(3) 209	(4) 214	(5) 273	(6) 280	(7) 259	(8) 260
1	1	1		0.99	0.12	0.41	0.00	0.00	0.00	0.00
1	1	2	0.99		0.45	0.86	0.00	0.00	0.00	0.00
1	2	1	0.12	0.45		0.99	0.00	0.00	0.00	0.00
1	2	2	0.41	0.86	0.99		0.00	0.00	0.00	0.00
2	1	1	0.00	0.00	0.00	0.00		0.98	0.52	0.68
2	1	2	0.00	0.00	0.00	0.00	0.98		0.11	0.17
2	2	1	0.00	0.00	0.00	0.00	0.52	0.11		0.99
2	2	2	0.00	0.00	0.00	0.00	0.68	0.17	0.99	

Wingate Lactate

Effect	Wilks' Lambda	Rao's R	DF 1	DF 2	p-level
1	0.58	6.25	2	17	0.01*
2	0.97	0.28	2	17	0.76
3	0.86	1.36	2	17	0.28
12	0.99	0.07	2	17	0.94
13	0.85	1.51	2	17	0.25
23	0.97	0.25	2	17	0.78
123	0.97	0.30	2	17	0.75

Wingate Lactate, Main effect of Sex

Variable	MS Effect	MS Error	F (DF 1, 18)	p-level
PRE	1	1	0.87	0.36
POST	157	12	13.03	0.00*

Dorsiflexor Fatigue Protocol

Effect	Wilks' Lambda	Rao's R	DF 1	DF 2	p-level
1	0.41	10.24	3	21	0.00*
2	0.84	1.37	3	21	0.28
3	0.89	0.84	3	21	0.49
12	0.82	1.53	3	21	0.23
13	0.71	2.87	3	21	0.06
23	0.98	0.13	3	21	0.94
123	0.89	0.86	3	21	0.48

Cybex Knee Extension (average of 6 contractions)

Effect	Wilks' Lambda	Rao's R	DF 1	DF 2	p-level
1	0.42	9.53	3	21	0.00*
2	0.95	0.34	3	21	0.80
3	0.91	0.67	3	21	0.58
12	0.93	0.54	3	21	0.66
13	0.79	1.90	3	21	0.16
23	0.89	0.89	3	21	0.46
123	0.89	0.91	3	21	0.46

Handgrip Test

Effect	Wilks' Lambda	Rao's R	DF 1	DF 2	p-level
1	0.14	19.08	6	18	0.00*
2	0.81	0.71	6	18	0.65
3	0.79	0.80	6	18	0.58
12	0.82	0.65	6	18	0.69
13	0.81	0.70	6	18	0.65
23	0.67	1.47	6	18	0.24
123	0.83	0.62	6	18	0.71

Handgrip Test, First 6 Contractions

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	10676	1	223	23	47.84	0.00*
2	15	1	223	23	0.07	0.80
3	435	5	13	115	34.38	0.00*
12	113	1	223	23	0.51	0.48
13	96	5	13	115	7.61	0.00*
23	10	5	13	115	0.77	0.58
123	6	5	13	115	0.45	0.81

Handgrip Test, Last 6 Contractions

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	11672	1	337	23	34.64	0.00*
2	0	1	337	23	0.00	0.97
3	537	5	11	115	47.32	0.00*
12	144	1	337	23	0.43	0.52
13	77	5	11	115	6.82	0.00*
23	7	5	11	115	0.62	0.69
123	1	5	11	115	0.12	0.99

Handgrip Test, Fatigue Percentage Relative to Initial Force

Effect	MS Effect	DF Effect	MS Error	DF Error	F-Value	p-level
1	1226	1	271	23	4.52	0.04*
2	0	1	271	23	0.00	0.98
3	9	1	146	23	0.06	0.81
12	69	1	271	23	0.26	0.62
13	2	1	146	23	0.01	0.91
23	268	1	146	23	1.84	0.19
123	141	1	146	23	0.97	0.34