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The Influence of Type II Muscle Fibers and Creatine Supplementation on Repeated Bouts of the Wingate Anaerobic Test

A Thesis Presented to

The School of Kinesiology,

Lakehead University

In partial fulfillment of the requirements for the

Degree of Master of Science

in

Kinesiology

Marc A. Poirier

April 18th, 2001

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ABSTRACT

Title of Thesis: The Influence of Type II Muscle Fibers and Creatine Supplementation on Repeated Bouts of The Wingate Anaerobic Test.

Thesis Advisor: **Dr. Norman LaVoie,**

Professor; Lakehead University.

Committee: **Dr. Robert Thayer,**

Associate Professor; Lakehead University.

Dr. Jim McAuliffe,

Associate Professor; Lakehead University.

Author: **Marc A. Poirier.**

The main purpose of this thesis was to investigate the influence of muscle fiber type composition and supplemental creatine monohydrate on repeated bouts of the Wingate Anaerobic Test (WAnT). More specifically, would a higher percentage of Type II muscle fibers demonstrate a greater significant improvement in repeated bouts of cycle ergometry after creatine supplementation. Nineteen males (mean ± SD age, body mass, and height = 21.7 ± 1.9 yr., 84.1 ± 14.1 kg and 161.5 ± 7.8 cm, respectively) participated in the 13 day experiment. Initially, sixty-five participants performed a single 30-s WAnT against a resistance of .10 kg/kg body weight. All scores were then rank ordered from highest to lowest, according to relative peak anaerobic power (PAPr) scores. Ten participants from both ends of this distribution were approached and requested to volunteer for the remainder of the study. This allowed the following four groups to be constructed: 1) high WAnT with creatine (n = 5), 2) high WAnT with placebo (n = 5), 3)

low WAnT with creatine (n = 5), and 4) low WAnT with placebo (n = 4). On days 4 and 12 of the study, percutaneous muscle biopsies were obtained from the vastus lateralis and stained histochemically to determine fiber type distribution (Type II, IIa, and IIb). This data demonstrated a positive, but weak relationship between peak anaerobic scores (relative) obtained during the single 30-s WAnT and percent type II muscle fibers ($r = 0.52$, $p<0.05$). Furthermore, a Spearman rank-order correlation revealed a positive monotonic relationship ($R = 0.611$, $p<0.01$) between the variables. Finally, a 2 x 2 factorial ANOVA (F (1,15) = 12.8, $p<0.01$), revealed that the 10 participants within the high PAPr group had higher values for %FT, regardless of treatment. This data revealed that the grouping of participants according to PAPr during the single 30-s WAnT was reflective of their fiber type distribution in regards to percent type II muscle fibers. Five repeated bouts of the WAnT were performed on days 5 and 12 of the study, with each bout lasting 15-s, against a resistance of 0.075 kg/kg body weight and with 45-s of active rest between each bout. Supplementation occurred on days 7 through 11, with groups # 1 and #3 receiving the treatment (4 x 5g of creatine + 2g of dextrose powder) and groups # 2 and # 4 receiving the placebo (4 x 7g of dextrose powder). Peak anaerobic power (absolute and relative), mean anaerobic power (absolute and relative) and percent power decrease were recorded during each of the five bouts. The design was a 2 (time: pre or post) by 2 (treatment: creatine or placebo) by 2 (WAnT: high or low) by 5 (bouts: 1 through 5) split-plot factorial analysis. Change scores were calculated for all five dependent measures and consequently the data was analyzed with a 2 (treatment: creatine or placebo) by 2 (WAnT: high or low) by 5 (bouts: 1 through 5) split-plot factorial ANOVA, which revealed no significant main or interaction effects. Furthermore, there

was a significant increase in body weight, from pre to post-supplementation, regardless of treatment. The results therefore, suggest no relationship between participants' fiber type distribution (through the stated relationship to PAPr), creatine supplementation, and repeated bouts of the WAnT. However, due to the small number of participants per cell or group within this study, the results should be viewed with caution.

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To the PFLC students who volunteered and helped gather the data.

To the participants, without you there would be no study.

Finally, to my parents and Laura, who have supported me in many ways during the past few years.

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CHAPTER 1

INTRODUCTION

Statement of the problem

The main purpose of this thesis was to investigate the influence creatine monohydrate supplementation and muscle fiber type composition on five repeated bouts of the Wingate Anaerobic test (WAnT). More specifically, did a higher percentage of Type II muscle fibers demonstrate a significant improvement during any or all bouts of the WAnT?

Significance of the study

In this day and age, athletic competition can place enormous pressure on those involved to be successful, and consequently, ergogenic aids are used by many amateurs and professionals. One particular ergogenic aid, creatine (Cr), has become a widely used nutritional supplement by both high caliber and recreational athletes. In fact, Bolotte (1998) stated that approximately 80% - 90% of professional football players and track and field athletes have used creatine. Furthermore, creatine is not currently banned by any major sport governing bodies, including the International Olympic committee, since it is not considered an illegal chemical substance (McArdle, Katch, & Katch, 1999). Consequently, creatine has become one of the most popular ergogenic aids in the sport supplement market over the past decade.

Normal total creatine resting values (creatine and phosphocreatine) are on average 124 $mmol \cdot kg^{-1}$ dry muscle (dm), with 95-98% located within skeletal muscle and the

remaining 5% found primarily in heart, brain, liver, kidney and testicle tissue (Balsom, Soderlund, & Ekblom, 1994; Ekblom, 1996; Harris, Soderlund, and Hultman, 1992).

The esticular importance of phosphocreatine (PCr) can be realized when one reviews its role in bioenergetics. Adenosine triphosphate (ATP) is the human energy currency and facilitates digestion, circulation, tissue synthesis, nerve conduction, glandular secretion, and muscular contraction (McArdle et al., 1999). An increase in creatine stores should allow for prolonged use of phosphocreatine in resynthesizing ATP anaerobically, which in turn decrease reliance on anaerobic glycolysis (McArdle et al.). This is beneficial to repeated bouts of intermittent, high intensity performance, as anaerobic glycolysis will produce lactic acid levels that result in a decrease in pH and ultimately affect performance in a negative manner.

Muscle fiber type distribution most likely regulates initial creatine levels and uptake for any given individual. Type II muscle fibers have a higher resting concentration of PCr than Type I muscle fibers and greater phosphocreatine utilization, with a slower rate of resynthesis of phosphocreatine during repetitive bouts of intermittent, high intensity exercise (Tesch, Thorsson, and Fujitsuka, 1989; Soderlund, Greenhaff, and Hultman, 1992). Also, Greenhaff, Bodin, Soderlund, and Hultman (1994) reported that Type II muscle fibers have approximately 45%-55% higher values of creatine than do Type I muscle fibers. Furthermore, Type IIb, fast twitch muscle fibers, have been shown to store 4-6 times more phosphocreatine than ATP (Casey, Constantin-Teodosiu, Howell, Hultman, and Greenhaff, 1996). These findings illustrate that muscle fiber type distribution plays an integral role in creatine uptake and utilization during exercise and therefore those individuals with a higher percentage of Type II muscle fibers would be

expected to outperform subjects with lower concentrations of Type II muscle fibers during repeated bouts of intermittent, high intensity exercise.

To date, previous studies investigating the uptake of creatine and repeated bouts of intermittent high intensity exercise, such as the WAnT, have not grouped participants according to fiber type, or in groups of high or low percentage of Type II muscle fibers. Positive correlations ranging from 0.54 to 0.81 have been reported between relative peak anaerobic power (PAPr) scores achieved during a single 30-s WAnT and muscle fiber type distributions (Bar-Or et al, 1980; Froese and Houston 1987; Inbar, Kaiser, and Tesch, 1981; Kaczowski, Montgomery, Taylor and Klissouras, 1982). Although the groups within this study were formed on the basis of PAPr, the aforementioned correlations should allow some conclusions to be drawn in regards to the role that fiber type distribution had in creatine supplementation and repeated bouts of the WAnT.

Delimitations

1) The original 65 male participants for this study were students at Lakehead University, ranging in age from 19 - 27 years old. The final 19 participants were selected on the basis of PAPr scores obtained during the single 30-s WAnT.

Limitations

1) It was assumed that at all times during the single and repeated bouts of the WAnT, all participants gave maximal effort.

2) The accuracy of participants in recording their nutritional intake during the study was a limiting factor.

3) All instruments used in the collection and analysis of data were in themselves limiting factors.

4) It had to be assumed that the manufacturer accurately described the purity and quality of the creatine monohydrate.

Definitions

Creatine is an organic compound obtained predominantly from ingestion of meat or fish, which contains ≈5 g of Cr· kg^{-1} (Volek & Kraemer, 1996). Furthermore, creatine is synthesized primarily within the liver, pancreas, and kidney by means of the precursor amino acids glycine, the guanidino group of arginine, and methyl group from S-adenosylmethionine (Murray, Granner, Mayes, & Rodwell, 1996).

Creatinine is formed through the degradation of creatine and phosphocreatine and is formed at a slow and constant rate through a nonenzymatic, irreversible reaction (Volek and Kraemer, 1996).

Mean Anaerobic Power (MAP) is the total number of revolutions of the flywheel during the performance, divided by 30 (30 seconds of total cycling). It is also defined as the total power output divided by 30 seconds, which can be expressed in relative (MAPr) or absolute (MAPa) terms (Inbar, Bar-Or, and Skinner, 1996). Mean anaerobic power is calculated as follows:

MP (Watts) = Total Work (Watts) / 30 (seconds)

Peak Anaerobic Power (PAP) is the maximum number of revolutions of the flywheel in a 5-s period. Is also defined as the maximum power output attained during 5-s

of cycle ergometry, which can be expressed in relative (PAPr) or absolute (PAPa) terms. (Inbar et al., 1996). Peak anaerobic power is calculated as follows:

PAP (Watts) = Force x Distance / Time [N x (Max Revs x 6 metres)] / 5sec

Phosphocreatine is the phosphorylated derivative of creatine found within muscle and is a high-energy compound that can reversibly donate a phosphate group to ADP to form ATP (McArdle et al., 1999).

Rate of Fatigue (Fatigue Index) is the numerical difference between the highest number of flywheel revolutions and the lowest number of flywheel revolutions, expressed as a percent of peak power (PP) (Inbar et al., 1996). Rate of fatigue is calculated as follows:

Rate of Fatigue = [(Peak Power (W) - Lowest Anaerobic Power (W)) / Peak Power] x 100

CHAPTER 2

LITERATURE REVIEW

Historic Overview

A historic overview of creatine (Cr) and its applications to athletic performance is a logical first step in discussing creatine supplementation. Creatine was initially discovered by the French scientist, Chevreul, in 1832 (Greenhaff, 1994). However, due to technological limitations in detecting creatine, no significant advances were made until 1847, when Lieberg confirmed that creatine was a regular component of muscle extracted from mammals (Greenhaff, 1994). Furthermore, Lieberg reported that muscle samples of wild foxes killed in chase contained ten times more creatine than captive animals and concluded that muscle activity resulted in an increase in creatine (Demant and Rhodes, 1999). In retrospect, this is understandable, as wild foxes would be prone to frequent bursts of sprinting during daily hunting sessions. Shortly thereafter, Heintz and Pettenkofer discovered a urinary product that Lieberg later confirmed to be creatinine, a metabolic end-product of creatine degradation (Balsom et al., 1994). From the observation that creatinine excretion was related to muscle mass, it was postulated that creatinine found in urine was directly linked to creatine levels within muscle (Balsom et al., 1994)

During early creatine feeding studies, Chanutin (1926) noted that not all ingested creatine could be accounted for in urine samples and speculation arose that some unspecified quantity of creatine was retained by the body (as cited in Greenhaff, 1995). The specific site of exogenous creatine uptake within the body was somewhat clarified in

1912 and 1914 by Folin and Denis, who reported that muscle levels increased by nearly 70% in cats that were fed creatine (Balsom et al., 1994).

The first attempt at quantifying the amount of creatine contained within man came about in 1923 by Hahn and Meyer, who estimated that total creatine content of a 70 kg male to be approximately 140g (Greenhaff, 1995). The next major development in creatine research came in 1927 when Fiske and Subbarow first reported phosphocreatine (PCr) as a "labile phosphorous" in resting cat muscle, with levels dropping during muscular electrical stimulation and reappearing during the ensuing rest period (Balsom et al., 1994). From this early research, it became evident that creatine and phosphocreatine were vital to energy metabolism and that an increase in resting creatine muscle levels could be advantageous to certain athletic events (Volek and Kraemer, 1996).

To aid in understanding the role of creatine in muscle metabolism, researchers began to rely on the muscle biopsy technique that Bergstrom implemented in 1962 while studying muscle electrolytes in man (Demant and Rhodes, 1999). Hultman, Bergstrom, and Anderson (1967) reintroduced the muscle biopsy technique to study the breakdown and resynthesis of ATP and PCr with exercise in humans. A more recent development in muscle metabolism research was the implementation of magnetic resonance spectroscopy (MNR) (Demant and Rhodes, 1999).

What is Creatine?

Creatine is a nitrogenous organic compound (methylguanidine-acidic acid), that can be thought of as a specialized product synthesized primarily in the liver, kidneys, and pancreas from the precursor amino acids arginine, glycine, and methionine (Murray,

Granner, Mayes, and Rodwell, 1996). In general, arginine will donate a guanidino group to glycine and form guanidoacetate, which is then methylated in the liver by S-adenosylmethionine, with the end result being the formation of creatine (Murray et al., 1996.) The role of creatine is to ultimately combine with a phosphate group to form phosphocreatine, which in turn is used to rephosphorylate adenosine diphosphate (ADP) to ATP (McArdle et al., 1999). Research has shown that a daily requirement of creatine (2g) is needed, either exogenously or endogenously, since it and phosphocreatine have been shown to degrade in an irreversible, nonenzymatic reaction at an approximate rate of 1.6% per day (Crim, Calloway, and Margen, 1976; Ekblom, 1996; Volek & Kraemer, 1996). As an example, a 70 kg individual would have a total creatine pool of 120 g and approximately 2 g would be replaced on a daily basis (Balsom et al., 1994). Creatine can be ingested by consuming fish and meats, which contain approximately 5 g $Cr \cdot kg^{-1}$, or as a nutritional supplement (creatine monohydrate) in a powder, tablet, capsule, or stabilized liquid form (McArdle et al.).

Role of Creatine in Exercise

Creatine is essential in the bioenergetics of ATP production via phosphorylation, the process of storing energy as ATP from other chemical sources (Wilmore & Costill, 1994). Unlike ATP, the energy derived from phosphocreatine is not used directly to accomplish work, but to rephosphorylate adenosine diphosphate (ADP) to ATP (Wilmore & Costill, 1994). The above process is aided by the enzyme creatine kinase (CK), which removes a phosphate from PCr and couples it to ADP, creating ATP (Wilmore & Costill, 1994). This energy system is known as the ***ATP-PCr*** system, which is presented below in

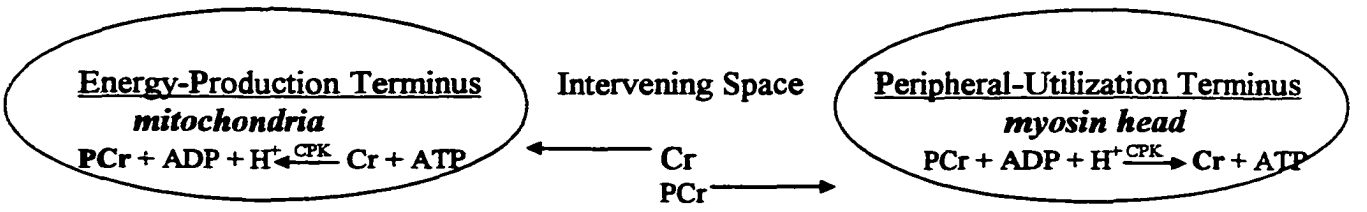
figure 1 and the key reaction responsible for the rephosphorylation of ATP is known as the ***creatine kinase reaction***.

Figure 1. Creatine kinase reaction

However, it is generally agreed upon by most researchers in exercise physiology, that creatine is an essential energy substrate only during very specific forms of exercise, such as short bursts of muscular activity (McArdle et al., 1999).

A second crucial role of creatine during high intensity exercise is to act as a spatial energy buffer through a process known as the "phosphocreatine energy shuttle" (McArdle et al., 1999). This process involves the diffusion of creatine and phosphocreatine between sites of utilization and production of phosphocreatine. This system is comprised of a peripheral terminus for phosphocreatine utilization (myosin head for muscle action), an energy production terminus located within the mitochondria, and an intervening space between the sites (Volek & Kraemer, 1996). The aforementioned process is best understood in the following diagram of figure 2.

Figure 2. Phosphocreatine Energy Shuttle



At the peripheral terminus, phosphocreatine donates its phosphate in order to rephosphorylate ADP, so that ATP demand is fulfilled and exercise can continue. In the process, creatine is liberated and travels back to the energy-production terminus via the intervening space (in the opposite direction of phosphocreatine) were it can than be utilized to produce more phosphocreatine, which will return to the peripheral terminus to rephosphorylate ADP and compete the shuttle (Volek & Kraemer, 1996).

A third reported beneficial function of creatine is its ability to buffer excess protons (H^+) produced during ATP production. Accumulation of lactate results in an increase in H^+, which will decrease the cell pH and performance of the muscle will be negatively affected. Fortunately, when the creatine kinase reaction is in the process of producing ATP, H^+ are utilized and a normal pH is maintained (Volek & Kraemer, 1996).

A fourth noted benefit of creatine is as a mediator of glycolysis during exercise. At the commencement of intense exercise, glycolytic demand can increase up to a hundred fold and phosphocreatine levels drop dramatically so that ATP may be rephosphorylated at the active muscle sites (Volek & Kraemer, 1996). Some in vitro research implies that low levels of phosphocreatine may play a role in stimulating glycolysis. It has been suggested

that phosphofructokinase (PFK) is conceivably inhibited at certain physiological concentrations of phosphocreatine, but that during strenuous exercise when phosphocreatine levels drop, PFK is activated and glycolysis is initiated with the end result of more ATP production for active muscles (Volek & Kraemer, 1996).

Muscle Fiber Types and Creatine

It is generally agreed upon that Type II muscle fibers have higher basal levels of phosphocreatine than in Type I muscle fibers (Tesch, Thorsson, and Fujitsuka, 1989). Furthermore, human studies have shown that creatine kinase activity is higher in type II than in type I muscle fibers (Jansson and Sylven, 1985; Thorstensson, Sjodin, Tesch, and Karlsson, 1977). This higher enzyme concentration in Type II muscle fibers most likely indicates that these muscle fibers do indeed have higher basal levels of phosphocreatine than do Type I muscle fibers. This could imply that individuals with higher concentrations of Type II fibers would retain more supplemental creatine and perform more favourably in intermittent, repeated bouts of maximal exercise than individuals with lower concentrations of Type II muscle fibers.

It is also important to note the relationship between power measurements obtained during exercise performance and fiber type distribution. Since its development in the 1970s, the Wingate Anaerobic Test (WAnT) has become the most widely used test in assessing muscular power, endurance and fatigability in humans (Inbar, Bar-Or, and Skinner, 1996). Furthermore, the WAnT is a simple, inexpensive and noninvasive technique, which is in direct contrast to the procedure of obtaining a muscle biopsy. In addition, power indices obtained from the WAnT have been positively correlated to leg

muscle fiber type composition (Inbar et al., 1981). For example, correlations of r = 0.54 and r = 0.57 were reported between PAP (WAnT) and percent Type II muscle fiber distribution (Bar-Or et al., 1980; Inbar et al., 1981). Furthermore, PAP and total work (TW) have also been correlated with fiber type distribution (percent Type II). Correlations of r = 0.59 (PAP), r = 0.81 (TW), and r = 0.81 (PAP), r = 0.62 (TW) were reported by Kaczkowski et al. (1982), and Froese and Houston (1987) respectively. Therefore, grouping participants according to PAPr scores obtained during a single 30-s WAnT should also reflect fiber type distribution according to these previously reported relationships.

Creatine Supplementation

Creatine supplementation, with the hope of augmenting athletic performance, is prevalent at all levels of organized sport. As previously stated, up to 80-90% of professional football players and track and field athletes have used creatine monohydrate in supplemental form at one point (Bolotte, 1998). Creatine supplementation made media headlines during the 1992 Summer Olympic Games, due to claims of the ergogenic effect of creatine supplementation by British sprinters and hurdles (McArdle et al., 1999). This is not surprising since creatine is essential for intermittent, repeated bouts of maximal exercise and has been shown to be retained in the body to some extent. Normal resting plasma concentrations of creatine are in the range of 50-100 μmol/L and ingesting 5 g of creatine in solution will raise plasma concentration levels to 600-800 μmol/L within 1 hour (Green, Hultman, Macdonald, Sewell, and Greenhaff, 1996; Harris et al., 1992). Creatine uptake into muscle cells occurs against a concentration gradient, by means of a family of

Na^+ dependent neurotransmitter transporters (Guimbal and Kilimann, 1993). Furthermore, increases in muscle creatine levels can be augmented by ingesting creatine in combination with carbohydrates (Green et al., 1996). It is generally accepted that 20 g/day of creatine (4 x 5 g doses/day) for 5 days will on average increase muscle creatine levels by 20%, of which 20% is in the phosphorylated form (Greenhaff, Constantin-Teodosiu, Casey, & Hultman, 1994; Harris, Soderlund, & Hultman, 1992). Similar results have been reported when 3 g/day for 28 days was utilized (Hultman, Soderland, Timmons, Cederblad, and Greenhaff, 1996). The greatest gains in total Cr have been in those individuals with the lowest initial values (118 $mmol \cdot kg^{-1}dm$) and there is speculation that vegetarians would have the largest gains in creatine uptake due to low initial stores (Balsom, Ekblom, Soderlund, Sjodin, and Hultman, 1993; Harris et al., 1992; Delanghe et al., 1989). The uptake of creatine appears most significant during the initial days of supplementation, as illustrated by Harris et al. (1992) when they reported 70% retention on day 1, but nearly 0% on day 7. This has led to the conclusion that there is an upper limit, or saturation point of creatine uptake, of approximately 160 $mmol \cdot kg^{-1}$ (Harris et al., 1992). However, the determining factors that regulate whether an individual has high or low concentrations of creatine in their muscle remains unknown and is in need of further research (Greenhaff, 1994).

Side Effects of Creatine Supplementation

To date, the only consistently reported side effect with short-term, oral creatine supplementation has been a statistically significant increase in body mass from 0.5 kg to 2.4 kg (McArdle et al., 1999). The largest increases have been in those who possess low initial values, most notable vegetarians (Harris et al., 1992). Reduced urinary volume during creatine supplementation suggests that an increase in body mass can be attributed to excess water retention (Hultman et al., 1996). Another possible cause for increases in body mass, is a significant augmentation in lean body mass after creatine supplementation. Six weeks of strength training and creatine supplementation (20g/day for 7 days, 2g/day thereafter) resulted in significant increases in fat free mass for 23 male participants (Becque, Lochmann, and Melrose, 1997). To date, it is accepted that water retention and increases in lean body mass are the most likely causes for the significant increases in body mass with creatine supplementation (Williams and Branch, 1998).

The purity of supplemental creatine has been called into question as a possible health risk and certain guidelines to be followed when purchasing creatine are available (McArdle et al., 1999). These same researchers claim that the increase in body mass could be from the anabolic effect of Cr on muscle synthesis, water retention within muscle cells, and/or other unknown factors. Furthermore, anecdotal reports from numerous athletic trainers suggest a possible link between creatine supplementation and muscle cramps, muscle spasms, and pulled muscles (Clarkson, 1998).

To a large extent, much of the controversy surrounding creatine supplementation came about with an anecdotal report that surfaced in December of 1997, in which it was

suggested that creatine supplementation was partially responsible for the deaths of three American collegiate wrestlers (Clarkson, 1998). As a result of creatine supplementation, these wrestlers were likely experiencing difficulty "making weight" due to excess water retention within muscle cells. This could have caused the wrestlers to use even more drastic means of losing weight than normally practiced and the excessive state of dehydration that can be induced most likely resulted in fatal thermal stresses (Clarkson, 1998).

Many researchers have noted a lack of information regarding the long-term side effects of creatine monohydrate supplementation. Juhn and Tarnopolsky (1998) provide an excellent literature review in which possible negative side effects of long-term creatine supplementation were listed. These included: cardiovascular, gastrointestinal/liver, musculoskeletal, neurologic, oncologic, pediatric/adolescent, renal (kidney), reproductive organs, weight gain, and dehydration. One of these aforementioned organ systems, the kidneys, has been cited in numerous articles related to creatine supplementation. It is theorized that the one carboxyl and two amino groups of creatine, as well as the high nitrogen content (32%) could place excessive and perhaps dangerous amounts of stress on the kidneys (Poortmans et al., 1997). More controversy surfaced when Pritchard and Kalra (1998) reported that a patient under their care presented "substantial" renal dysfunction while taking oral creatine supplementation (15g/day for 7 days, 2g/day for 7 weeks thereafter). The individuals' renal functions returned to within the normal range only after creatine supplementation ceased (Pritchard and Kalra, 1998). However, it must be noted that this report involved only a single participant, who was suffering from focal

segmental glomerulosclerosis for 8 years before creatine supplementation. A more recent and unsettling development pertains to a report by the French Food Safety Agency, in which they claim that long-term oral creatine supplementation constitutes "a potential carcinogenic risk" ("Creatine linked," 2001).

Creatine Supplementation Research

A table format was utilized on the following pages to present to the reader a simplistic means of reviewing literature related to creatine supplementation and athletic performance. Forty-three (43) articles were reviewed in hopes of determining why specific findings were reported, with the following categories being used: creatine dosage, performance protocol, measurements for analysis, participant pool, results, and reasons for findings. Each article was categorized as being either "statistically significant" or " statistically nonsignificant" in terms of the results. Although not all-vital information was contained within these tables, it should provide a quick reference source for any individual interested in creatine supplementation research to date.

Table 1. Nonsignificant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Barnett et al., 1996	4 x 70 mg/kg^{-1} body mass Cr/day mixed with 5g glucose for 4 days	•7 x 10s cycle ergometer •30s recovery for sprints 1-5, 6-7 •5 min. recovery for sprints 5-6	•peak power •mean power •end-power output •% power decline	•10 active males	•Cr had no influence on any parameter	•Cr dosage did not elevate resting muscle Cr levels? •only speculation as no tissue, blood or urine was collected
Febbraio et al., 1995	•20g Cr/day for 5 days •crossover design with 4 week washout period	•4x60s cycle ergometry with 60s rest at 115-125% $V0_2$ max •1 exhaustive sprint	•adenelytes •ammonia •PCr •Cr •glycogen •blood lactate	•6 active & untrained males	•Cr had no influence on any parameter	•duration of exercise is not heavily influenced by the ATP/PCr pathway
Cook et al., 1995	4 x 5g Cr + 1g glucose for 5 days	•1 x 15s cycle ergometry •20 min. rest •1 x 15s cycle ergometry •111.8N resistance for all subjects	•peak power (pp) •time to pp •total work •fatigue index	•12 healthy untrained men	•no significant differences between or within groups	•free Cr increased by PCr did not? •only speculation as no tissue, blood or urine was collected
Odland et al., 1997	20g Cr/day for 3 days	•3 randomly ordered wingates •14 days apart •creatine •placebo •control	•peak power •10s power •30s power •% fatigue •post-exercise blood lactate levels	•9 healthy males	•Cr had no effect for any measured parameter	• Cr dosage did not elevate resting muscle Cr levels? •only speculation as no tissue, blood or urine was collected
Balsom, Harridge, et al., 1993	4x5g Cr+1g glucose for 6 days	•exhaustive treadmill run •6km terrain run	•performance time •$V0_2$ & heart rate •blood lactate •hypoxanthine	•18 habitually active to well-trained males	•Cr had no effect for any measured parameter •↑ 6 km time	•Cr (ATP/CP sys) is not a major energy pathway for exercises in excess of 30s

Table 2. Nonsignificant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Thompson et al., 1996	2g Cr/day for 6 weeks	•plantar flexion •10-15min in duration	•PCr/ATP •Ph •PCr/ADP •Q_{max} • Pcr $t_{1/2}$ •Reoxygenation $t_{1/2}$	10 highly trained female swimmers	Cr had no effect for any measured parameter	•exercise protocol was not intermittent, high intensity in nature? •exogenous Cr suppressed endogenous Cr production?
Mujika et al., 1996	4x5g Cr/day for 5 days	•swimming test •25m,50m,&100m •20-25min rest periods	•blood lactate •blood ammonia •performance time	•20 highly trained swimmers • 9 females •11 males	•significant ↑ in bw •significant ↓ in post 50m&100m ammonia levels (Cr) • significant ↓ in post 50m ammonia levels (Pl) •Cr had no other effect	•participants were elite and perhaps already at upper limits in terms of resting muscle Cr levels? •↑ in bw resulted in slower performance time?
Earnest, Rash et al., 1995	•20g Cr/day for 4 days •then 10g Cr/day for 6 days	•2x treadmill runs until exhaustion •runs lasted around 90s and 8 min recovery period	•blood lactate •performance time	11 male subjects	Cr had no effect for any measured parameter	•participants were elite and perhaps already at upper limits in terms of resting muscle Cr levels? •↑ in bw resulted in slower performance time? • rest period sufficient for CP resynthesis regardless of supplementation?

Table 3. Nonsignificant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Redondo et al., 1996	•25g mix of Cr and glucose for 7 days •83% of mix Cr	•3x60m runs •2min rest periods	•velocity (m/s)	•32 subjects •highly trained mixed athletes •14 females •8 males	Cr had no effect for any measured parameter	•more trials needed to induce a state of fatigue? • rest period sufficient for PCr resynthesis regardless of supplementation? •Cr dosage did not ↑ resting muscle Cr levels
Odland et al., 1994	•20g Cr/day for 3 weeks •crossover design •14 washout phase	•1x wingate cycle test	•peak power •mean 10s power •mean 30s power •% fatigue • blood lactate	9 male subjects	Cr had no effect for any measured parameter	•exercise protocol was not intermittent, high intensity in nature? •Cr (ATP/CP sys) is not a major energy pathway for exercises in excess of 30s •more trails needed to fully induce a state of fatigue? •Cr dosage did not elevate resting muscle Cr levels?
Rossiter et al., 1996	5x.25g Cr/kg body mass/day for 5 days	1000m simulated rowing performance	•creatine uptake (% of intake) •creatine uptake (g kg BM^{-1}) •performance time	•38 competitive rowers •28 males •10 females	•Cr had no effect for performance time ($r = 0.426$)	•exercise protocol was not intermittent, high intensity in nature? •Cr (ATP/CP sys) is not a major energy pathway for exercises in excess of 30s
Burke et al., 1996	4x5g Cr+2g glucose for 5 days	•**swimming test** •25m,50m,&100m •10min rest periods •**bike test** •2x10s cycle ergometry •10min rest	• **swimming test** •performance time •blood lactate •**bike test** •peak power •work done •time to peak	•32 elite swimmers •14 females •18 males	Cr had no effect for any measured parameter	•Cr dosage did not elevate resting muscle Cr levels? •participants were elite and perhaps already at upper limits in terms of resting muscle Cr levels? •unfamiliar rest period (psychological effect)?

Table 4. Nonsignificant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Terrillion et al., 1997	20g Cr/day for 5 days	•2x700m runs •60min rest	•performance time •blood lactate •body weight	12 well-trained & competitive male runners	Cr had no effect for any measured parameter	•89% of ↑ in Cr pool was free Cr • rest period sufficient for CP resynthesis regardless of supplementation?
Rawson et al., 1999	•20g Cr + 28g dextrose powder for 10 days •Continued by 4g Cr + 6.8g of dextrose for following 20 days • 1 serving of Gatorade™ ingested 30-min after ingestion of supplementation	•3 maximal isometric contractions of elbow flexors with 60-s rest •5x30 knee extensions @ 180° •1x30 max contractions •underwater weight to determine body composition	•Body mass •Body composition •Arm isometric strength •Leg fatigue •Peak fatigue	•20 healthy male participants •elderly (60-82 yrs.)	•Cr had no significant effect on all measured parameters	Uptake of Cr into muscle cell impeded due to age of participants?
Francaux et al., 1999	•21g Cr/day for 5 days then 3g Cr/day for 58 days •9 weeks of supplementation in total	•6-week training program •week1: 6x6 squats at 30% of max •workload ↑ to last week which had 8x6 squats at 42.5% of max •3-weeks of supplementation, but no training	•body water content •isokinetic force • single squat used for data analysis	•25 healthy males	•no significant improvements in any of the parameters after 6 weeks (42 days) or additional 3 weeks (days 42-53)	•exercise protocol was not intermittent, high intensity in nature?

Table 5. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Earnest et al., 1994	4 x 5g Cr + 1g glucose for 14 days	3x3 wingate test with 5min rest periods	•anaerobic capacity •peak anaerobic power •plasma ammonia	8 weight trained males	↑ in anaerobic capacity with Cr	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate?
Earnest, Snell, et., 1995	**wingate:** 20g Cr/day for 14 days **muscular strength:** 20g Cr/day for 28 days	**wingate:** 3 wingate test with 5min rest periods **muscular strength:** •1 RM bench press •repetitions of 70% of 1 RM bench press	**wingate:** •kJ during the 3 test **muscular strength:** 1 RM bench press •absolute (kg) •relative (kg kg^{-1}) Lifting reps-70% 1RM •number of reps •absolute (kg) •relative (kg kg^{-1})	•8 males •at least 10 years experience in weight training	**wingate:** ↑ in work done (kJ) for all 3 tests **muscular strength:** 1 RM bench press •↑ absolute Lifting reps-70% 1RM •↑number of reps •↑absolute •↑relative	• Cr dosage did elevate resting muscle Cr levels •exercise protocol was appropriate? •appropriate participant pool?
Harris et al., 1993	6x5g Cr + 5g glucose/day for 5 days	•4x300m runs- 3min rest •4x1000m runs - 4min rest	•performance times	•10 trained middle distance runners	significant ↓ in •final 300m •total 4x1000m •final 1000m	• Cr dosage did elevate resting muscle Cr levels? •4 x 300m appropriate •other results questionable

Table 6. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Schneider et al., 1997	**test 1**: 4x5g Cr + 1g glucose/day for 7 days **test 2:** additional 2 days of Cr supplementation	**test 1:** •5x15s cycle ergometry 1min rest period **test 2:** •5x60s cycle ergometry 5min rest period	•blood lactate •work (kJ) •total work (kJ) •% decrement in work	•9 untrained participants	•significant ↑ in work performed during all 5 bouts of 15s (Cr group) •significant ↑ in total work performed during all 5 bouts of 15s (Cr group) •lower post-exercise blood lactate (Cr group)	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participant pool?
Kreider et al., 1998	15.75g Cr/day for 28 days	•12x6s cycle ergometry 30s rest periods •bench press •squats •power clean	•numerous blood variables •body weight, water, & composition •performance time •blood lactate •body weight •work (J) •lifting volume	28 NCAA division IA football players	greater gains for Cr group in •fat/bone-free mass •isotonic lifting volume •sprint performance	•Cr dosage did elevate resting muscle Cr levels after 28 days? •exercise protocol was appropriate?
Van Leemputte et al., 1999	• 4x5g Cr/day for 5 days	•12 maximum elbow flexions •10-s rest periods	•Maximum torque •Contraction time •Relaxation time	•16 male physical education students	•Cr loading ↓ relaxation time needed between max. elbow flexions	•exercise protocol was appropriate? •Cr dosage did elevate resting muscle Cr levels?

Table 7. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Greenhaff et al., 1993	4x5g Cr+1g glucose/day for 5 days	•5x30s maximal voluntary isokinetic contractions •1-min rest periods	•muscle torque •ammonia •blood lactate	•12 physically active, but non-highly trained subjects •3 females •9 males	•↑ muscle torque for contractions (Cr group) •21-30 of bout 1, all contractions for bouts 2, 3 & 4 & 11-20 of bout 5	• Cr dosage did elevate resting muscle Cr levels •exercise protocol was appropriate •appropriate participant pool
Jacobs et al., 1997	20g Cr/day for 5 days	cycle ergometry until exhaustion •125% $V0_2$ max	•maximal accumulated 0_2 deficit •time to exhaustion	26 participants (mixed males & females)	•↑ in time to exhaustion (Cr group) • maximal accumulated 0_2 deficit ↑ (Cr group)	• Cr dosage did elevate resting muscle Cr levels? • findings are contrary to most involving the lactic acid energy system & exercise lasting 30-150-seconds
Lemon et al., 1995	•20g Cr/day for 5 days •crossover design •5 week washout	•20x30s maximal isometric ankle contractions •16s rest periods	•total integrated force •maximal force •body weight •pre-exercise muscle PCr/ATP ratio	•7 active male subjects	•↑ in total integrated force & maximal force after Cr •6/7 responders •↑ in body weight and pre-exercise muscle PCr/ATP ratio	• Cr dosage did elevate resting muscle Cr levels •exercise protocol was appropriate? •appropriate participant pool?

Table 8. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Prevost et al., 1997	•5 x 3.75g Cr/day for 5 days (18.75 g) •1 x 2.25g Cr/ day for next 6 days (13.5g)	•4 cycling protocols to exhaustion **A)** continuous **B)** repeated 30s, with 60s rest **C)** repeated 20s, with 40s rest **D)** repeated 10s, with 20s rest	•total work times •plasma lactic acid	•physically active & healthy subjects •10 males & 8 females	•Cr ↑ time to exhaustion (total work output) regardless or work protocol •plasma lactate accumulation ↓ following Cr	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participant pool?
Dawson et al., 1995	4x5g Cr + 1g glucose + 0.2g calcium carbonate for 5 days	**study 1:** 1x10s cycle ergometry **study 2:** •6x6s cycle ergometry •24s rest periods	**study 1:** •work done (kJ) at 2s, 4s, 6s, 8s, & 10s •total ($J \cdot kg^{-1}$) •peak power (W) •peak power ($W \cdot kg^{-1}$) •blood lactate and pH **study 2:** •Work done (kJ) for rep 1,6 and total work • total ($J \cdot kg^{-1}$) •peak power (W) •peak power ($W \cdot kg^{-1}$) •% decrement •blood lactate and pH	**study 1:** 18 healthy, active males **study 2:** 22 healthy, active males	**study 1:** Cr had no effect for any measured parameter **study 2:** •total work ↑ ($J \cdot kg^{-1}$ and kJ) •peak power↑ •work done in repetition 1 ↑ •Peak Power ↑ (W)	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate only in study two (repeated bouts) •appropriate participant pool

Table 9. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Balsom, Ekblom, et al., 1993	5x6g Cr+1g glucose/day for 6 days	•10x6s cycle ergometry •30s rest periods •resistance was 130rev/min or 140rev/min •24 hrs separated the above protocols	•blood lactate •hypoxanthine •oxygen uptake •exercise performance	•16 highly motivated male physical education students	•blood lactate ↓ for Cr group for both 130rev/min and 140rev/min •hypoxanthine ↓ during 140rev/min •significant between-group difference after 7th bout (higher pedal rev/min for Cr group) •significant ↓ in oxygen uptake for 130rev/min	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participant pool?
Birch et al., 1994	4x5g Cr/day for 5 days	•3x30s cycle ergometry •4min rest periods •80rpm	•peak power - PP (W) •mean power - MP (W) •work done ($J \cdot kg^{-1}$) •ammonia •blood lactate	14 healthy males	•significant ↑ in PP bout 1 •significant ↑ in MP bouts 1 and 2 •significant ↑ in work done during bouts 1 and 2	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participant pool?

Table 10. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Balsom et al., 1995	20g Cr/day for 6 days	**Part 1:** •5x6s cycle ergometry •30s rest periods **Part 2:** •40s after bout 5 of part 1 •1x10s cycle ergometry **Part 3:** •series of counter movement jumps and jump squats •before & after administration period	•blood lactate • plasma hypoxanthine •ATP •PCr •Cr •muscle lactate •body mass	7 highly motivated male participants	•↑ in body weight •Cr group better able to maintain power output during **Part 2** •↑ in TCr levels following supplementation •PCr ↑ and muscle lactate ↓ after bout 5 of **Part 1**, following supplementation	•no control group? • Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate?
Volek et al., 1999	25g Cr/day for 7 days	• 4 sets x 10 reps of jump squat @ 30% of 1 RM (2 min. rest periods) • 1 set bench press to fatigue @ 80% of 1 RM •10 min. rest period between jump squats and bench press	•Peak Power •Body composition •Muscle fiber type •Training quality	• 19 healthy males familiar with resistance training	•Cr a significant effect on all parameters.	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate?

Table 11. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Lawrence et al., 1997	4x60mg/kg body mass of Cr	2500 rowing ergometer	•performance time •body mass •$V0_2$ max •peak aerobic power •total $V0_2$ •blood lactate & pH	•trained rowers •minimum of 2 years competitive rowing and 2 months in training •10 females & 10 males	•significant ↓ in performance time for Cr group •no significant difference within groups found	• Cr dosage did elevate resting muscle Cr levels? • findings are contrary to most involving the lactic acid energy system & exercise lasting 30-150-seconds
Soderlund et al., 1994	20g Cr/day for 6 days	•5x6s cycle ergometry •30s rest periods •6th bout: 10s •140 rev/min	•Total Cr (free Cr + PCr) •body mass •muscle lactate •time at target speed	• 8 male participants	•better able to maintain target speed at end of 10s bout of cycle ergometry • ↑ Total Cr stores & bm •↓ La & ↑ PCr after 5th bout	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? • No control?
Theodorou et al., 1999	•25g Cr/day for 4 days, then •5g Cr/day for 2 months	•Interval swim sessions A: 10x50 m B: 8x100 m C: 15x100m •Varying rest intervals	•Performance time •Mean interval time	•22 elite male and female swimmers	•Significant ↑ in performance after 1st Cr loading phase •Unchanged after 2nd Cr loading phase in comparison to results after 1st Cr loading phase	• Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? • findings are contrary to most involving the lactic acid energy system & exercise lasting 30-150-seconds

Table 12. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Urbanski et al., 1999	•20g Cr/day for 5 days •double blind counterbalanced crossover design	•maximal & 3 submaximal bouts of isometric knee extensions & hand grips (5-min rest)	•isometric strength •time to fatigue	•10 physically active, untrained males	•Cr group significantly ↑ maximal isometric strength for knee extensions •significant ↑ time to fatigue during all 3 bouts of knee extensions	•Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participants? • Cr only influential on large muscle mass?
Stone et al., 1999	•creatine, creatine +calcium pyruvate, calcium pyruvate, or placebo •all received supplementation at 0.22g/kg/day for 5 weeks	•1 rep max of parallel squat & bench press •2 static vertical jumps 15 x 5-s cycle ergometer with 60-s rest between each	•body composition •maximal strength •net peak force •peak power •average peak power •total work	•42 collegiate football players	•Cr and Cr + calcium pyruvate ↑ body composition, maximal strength, peak power, average peak power, & total work	•Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participants?
Peyebrune et al., 1998	•3x3g Cr+1.5g maltodextrine+1.5 g glucose/day for 5 days	•1x50 yard swim •8x50 yard swim with 90s rest period	•performance times •heart rate •Blood lactate •Ammonia •Blood pH	•14 male university club swimmers	•no metabolic differences between groups (blood & urine) •↓ in time for repetitions 1-8	•8x50 yard swim with 90s rest period exercise protocol was appropriate? •Cr dosage did elevate resting muscle Cr levels? •appropriate participants?

Table 13. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Peeters et al., 1999	Creatine, phosphocreatine & placebo at 20g Cr/day for 3 days, then 10g Cr/day for 6 week period	.• testing was 1 RM bench press, leg press & preacher curl testing at pre, 3 & 6 weeks	•strength •body composition •blood pressure	•35 males	Significant influences on body composition & bench press strength	•Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participants?
Vukovich et al., 1999	•20g Cr/day for 5 days then 10g Cr/day for 16 days	•5x10-s sprint cycle ergometry with 40-s rest •1RM leg press, extension, & curl •30-min rest •5x30 max reps with 45-s rest between sets	•power output •PAP •MAP •fatigue index •peak torque •total work	•48 males actively involved in weight training for at least 6 months	•Cr had significant influence on all parameters	•Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participants?
Pearson et al., 1999	•5g Cr/day for 10 weeks	•1 RM bench press, squat, & power clean •maximal cybex power test	•body mass •strength •power	•16 male college football players	•Cr had no significant influence on body composition • Cr had a significant effect on strength, power, & body mass	•Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participants?
McKenna et al., 1999	• 6x5g Cr + 5 g dextrose/day •4 week washout period provided	•5x10-s maximal cycle ergometer sprints • rest intervals of 180-s, 50-s, 20-s, & 20-s.	•PAP •power output •cumulative work output •fatigue index •ATP, Cr, & PCr	•14 healthy, moderately active males & females	•Cr ↑ TCr, Cr, & PCr • No effect on performance	•Placebo effect? •Small sample size?

Table 14. Significant effect of creatine supplementation on exercise performance.

STUDY	Cr DOSAGE	PERFORMANCE PROTOCOL	MEASUREMENTS FOR ANALYSIS	PARTICIPANT POOL	RESULTS	REASONS FOR FINDINGS
Kirksey et al., 1999	•0.3g/kg/day for 6 weeks	•2 countermovement vertical jump •2 static vertical jumps with 1-min rest •5x 10-s max sprints	•vertical jump height •power output •peak power •average power •total work •power production •lean body mass •fatigue rates	•36 males & females	•significant improvements for: •jump height •cycle peak power •cycle total work •cycle power production •lean body mass	•Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate? •appropriate participants?
Stout et al., 1999	•5.25g Cr + 1g CHO or 5.25 g Cr + 33g CHO •4 times/day for 6 days	•pedaling at 70 rpm then adding appropriate power output at 2-3-s •terminated when 65 rpm could not be maintained	•body weight •anaerobic working capacity	•26 healthy males	•no significant changes on body composition •significant influence on AWC	•Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate •appropriate participants?
Kamber et al., 1999	•20g Cr/day for 5 days •double blind cross-over design	•10 x 6-s cycle ergometry sprints with 30-s rest periods	•blood lactate •Cr & creatinine levels in blood & urine •muscle volume	•10 well trained sport students	•significant ↑ in sprints (2 - 4 sec) for bouts 4 -7 and (4 - 6 sec) for bouts 8-10. • ↑ body weight •significant influence on lactate & Cr concentration	•Cr dosage did elevate resting muscle Cr levels? •exercise protocol was appropriate?

In reviewing the articles of "statistically nonsignificant", it appeared that one or more of the aforementioned categories contained inappropriate procedures. For example, several studies had appropriate performance protocols, but the creatine dosage was likely inadequate to significantly raise resting muscle creatine and phosphocreatine levels (Odland et al., 1994; Cooke, Grandjean, & Barnes, 1995; Barnett, Hinds, & Jenkins, 1996). Furthermore, if the creatine dosage was satisfactory, then other design flaws were apparent, such as, inappropriate participant pools. That is, inappropriate participant pools (elite anaerobic athletes and elderly participants) were utilized in some studies (Balsom, Harridge, Soderlund, Sjodin, and Ekblom, 1993; Earnest, Rash, Snell, Almada, and Mitchell, 1995; Mujika, Chatard, Lacoste, Barale, and Geyssant, 1996; Redondo, Dowling, Graham, Almada, and Williams, 1996). Also elite anaerobic athletes, through years of high caliber training, are likely near the creatine saturation point and would gain little to no benefit through creatine supplementation (Harris et al., 1992). As well, elderly participants are thought to have a decreased ability to uptake creatine into their muscle in comparison to younger participants (Rawson, Wehnert, and Clarkson, 1999). Furthermore, unsuitable performance protocols were included in some studies. Specifically, excessive exercise duration (i.e. > 30 seconds), rest periods sufficient in time for phosphocreatine resynthesis regardless of creatine supplementation (i.e. > 120-180 seconds), or insufficient number of bouts of exercise were utilized (Earnest et al.1995; Redondo, Dowling, Graham, Almada, and Williams, 1996; Rossiter, Cannell, and Jakeman, 1996; Terrilion, Kolkhorst, Dolgener, and Joslyn, 1997; Thompson et al., 1996).

The same aforementioned categories were also scrutinized when reviewing articles with "statistically significant" findings. However, it would appear that the research design was appropriate in each of the categories. A creatine dosage regiment of approximately 20g/day for 5 days was widely used and most certainly raised resting muscle creatine and phosphocreatine levels (Birch, Noble, and Greenhaff, 1994; Greenhaff et al., 1993; Soderlund, Balsom, and Ekblom, 1994). Participants were most often not highly trained and this would allow for greater uptake of the creatine (Lemon et al., 1995; Peyrebrune, Nevill, Donaldson, and Cosford, 1998). Furthermore, a majority of the studies incorporated performance protocols that consisted of repetitive bouts of intermittent, high intensity exercise (Dawson et al., 1995; Earnest, Snell, Rodriguez, Almada, and Mitchell, 1995; Scheinder, McDonough, Fadel, and Berwick, 1997).

It would appear that creatine supplementation is an effective ergogenic aid for the following reasons: 1) allows faster ATP turnover rate in maintenance of power output during short-term muscular effort, 2) delays phosphocreatine depletion, 3) delays excessive reliance on anaerobic glycolysis and therefore decreases formation of lactic acid, and 4) facilitates recovery from repeated bouts of intermittent, high intensity exercise via an increased rate of ATP and PCr resynthesis and thus allows for high-level power output (McArdle et al., 1999). Further research is needed to fully understand those circumstances in which creatine supplementation would be advantageous to athletic performance. One such circumstance is to examine the influence of muscle fiber type composition and supplemental creatine monohydrate and the effect on repeated bouts of the WAnT.

CHAPTER 3

METHODOLOGY

Purpose

This study was undertaken to determine the role that muscle fiber type and creatine supplementation have on repeated bouts of the Wingate Anaerobic Test (WAnT).

Participants

For the purpose of this study, 65 students from Lakehead University were recruited to perform a single, 30-s WAnT. All participants reported to the Lakehead University exercise physiology laboratory in clothing appropriate for vigorous exercise, having been previously instructed not to consume food or drink (except water) for 3 hours, nor to smoke within 2 hours of testing. Furthermore, no heavy exercise during the 24 hours leading up to testing was to be undertaken. Finally, all participants read a detailed cover letter (Appendix B) and signed an informed consent form (Appendix C) before any data collection occurred. Final approval by the Ethics Advisory Committee at Lakehead University was obtained before the commencement of this study. Refer to Appendix A for a simplistic outline of the following methodology that was provided to the participants.

Single 30-s WAnT

Body weight, height, blood pressure, and heart rate were recorded, and a PAR-Q (Physical Activity Readiness Questionnaire) administered upon arrival at the laboratory. This was followed by five minutes of slow, steady pace cycling, interspersed with bouts of

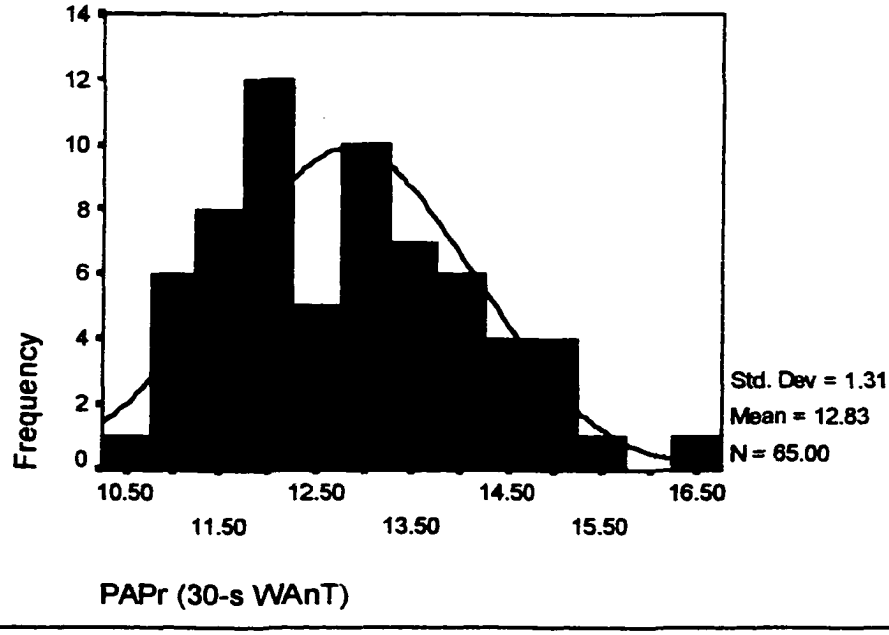
moderate sprinting. Furthermore, participants engaged in 5-minutes of lower body stretching, which emphasized the quadriceps, hamstrings, and calf muscles and an additional 5-minute rest period was given. Therefore, a preparation period of approximately 15-minutes was allowed for each participant before any bout of cycle ergometry. At the conclusion of the 15-minute preparation period, each participant had the handlebars, toe-straps, and seat height adjusted. The test instrument was a Monark cycle ergometer (824 E with toe straps) modified with a preloaded weight pan that could be applied directly to the flywheel. Individual resistance was calculated at 0.1 kg/kg body weight and the corresponding weight added to the pan. Testing commenced by cycling with maximal exertion against minimal resistance for no longer then 5 seconds, as this allowed the highest revolutions of the flywheel to be achieved. A 5-s countdown was provided and at time "0", the weight pan was applied to the flywheel for a period of 30 seconds and data recording began. The number of flywheel revolutions were recorded with an infrared counting system (Optosensor 2000 ^{TM}) and the computer software package EXTEND^{TM} determined relative and absolute peak anaerobic power (PAP), relative and absolute mean anaerobic power (MAP) and rate of fatigue (RF). The researcher was not involved in the data collection of WAnT scores and from this point in time, the study was conducted in a double-blind fashion.

Selection of treatment and placebo groups

At a later date, participants were rank ordered from highest to lowest, according to relative peak anaerobic power (PAPr) scores obtained during the single 30-sWAnT.

The purpose of this step was to construct a large distribution that represented PAPr scores, from which the final 20 participants were to be selected (see Figure 3). More specifically, 10 participants from both ends of this distribution were approached and requested to volunteer for the remainder of the study. Participants were selected in this manner due to the relationship that has been reported between power measurements during WAnT and muscle fiber type distribution (Bar-Or et al., 1980; Inbar et al., 1981; Kaczcowski et al., 1982; Froese and Houston, 1987). Unfortunately on the first day of data collection, one of the participants removed himself from the remainder of the study and the following four groups were constructed: 1) high WAnT with creatine ($n = 5$), 2) high WAnT with placebo ($n = 5$), 3) low WAnT with creatine ($n = 5$), and 4) low WAnT with placebo ($n = 4$)

Figure 3. Distribution and frequency of PAPr scores (30-s WAnT) for original 65 participants



Nutritional Assessment

Once participants were selected, they attended an information session in which all vital information related to their involvement in the study was presented, questions or concerns answered, and consent forms signed. The study began with participants maintaining a nutritional log (Appendix F) for 3 days before pre-supplementation muscle biopsies (days 1 through 3 of the study), and as well for a 3 day period before post-supplementation muscle biopsies (days 8 through 10). Participants consumed their regular diet and meticulously recorded all food consumed. This information was entered into Diet Analysis Plus (Version 4.0), which allowed pre and post-supplementation protein intake to be calculated. If any significant differences were detected in protein consumption between pre and post-supplementation, then those participants would be removed from the study. As previously stated, creatine can be formed endogenously through the combination of three amino-acids (Murray, Granner, Mayes, and Rodwell, 1996) and therefore it was imperative that this factor be controlled.

Repeated bouts of the WAnT

All 19 participants performed 5 repeated bouts of the WAnT on days 5 and 12 of the study. Each bout lasted 15-s in duration, with 45-s of active rest against a resistance of 0.075 kg/kg body weight. All other aspects of the repeated WAnT were identical to those previously describing the single 30-s WAnT within the methodology (page 33).

Muscle Biopsies and Fiber Type Determination

Pre and post-supplementation percutaneous muscle biopsies were obtained from the vastus lateralis muscle group according to the technique outlined by Bergstrom (1962). A medical doctor (Appendix D) using a 6 mm-biopsy needle, removed approximately 50-75 mg of tissue from each participant. Samples had all visible blood and connective tissue removed, oriented on a cork surface, fixed with OCT compound and then immediately frozen in 2-methylbutane, pre-cooled with liquid nitrogen (Brooke and Kaiser, 1970). Samples were then stored in an ultra-low freezer at approximately –80 °C until such time that analysis could occur. Muscle tissue stored upon cork was cut in serial sections at approximately -16° C, each 12 μm in thickness, and subjected to the myosin-ATPase staining technique after a 24 hour drying period (Brooke and Kaiser, 1970). Pre-incubation pH's of 4.30, 4.54, and 10.3 were utilized to achieve differentiation in identifying Type I, IIa, and IIb, muscle fibers for our specimens. The histochemical staining allowed fiber type composition to be calculated (% Type I, % Type II, % Type IIa, and % Type IIb).

Supplementation

Participants within the creatine groups were supplied with 20 bags of creatine monohydrate plus a carbohydrate additive (dextrose powder). Therefore, each bag contained 5 g of creatine monohydrate (EAS pure creatine) and 2 g of dextrose powder that was consumed 4 times per day for 5 days, beginning on day 6 and continuing until day 10 of the study. The placebo groups received the same number of supplements, with each bag containing 7 g of dextrose powder. No difference in appearance or flavour could be

detected between the bags containing the treatment and placebo. All participants had been instructed to consume their supplements at regular intervals throughout the day, at least 2 hours before or after a meal and/or exercise, in order to reduce the insulin response produced by feeding and exercise (Harris et al., 1992). Finally, participants recorded their nutritional intake on days 8-10 and returned to the laboratory on day 11 and 12, at which time post-supplementation muscle biopsies were taken and repeated bouts of the WAnT occurred. A questionnaire was provided on day 12 to all participants, which attempted to discover their level of compliance during the study (Appendix E).

Statistical Analysis

The design of the study was a 2 x 2 x 2 x 5 split-plot factorial ANOVA. More specifically, 2 (Time: pre and post) by 2 (Treatment: creatine or placebo) by 2 (WAnT: low or high) by 5 (Bouts: 1 to 5). The dependent measures during the five repeated bouts of the WAnT were relative and absolute PAP, relative and absolute MAP, and Fatigue Index (FI). To facilitate analysis, change scores were calculated for all dependent measures (pre minus post) during the five bouts. This resulted in 5 factorial ANOVA's being utilized to analyze the relationships between Treatment (main effect A), WAnT (main effect B), Bouts (main effect C), and the interaction effects of Treatment x WAnT, Treatment x Bouts, WAnT x Bouts, and Treatment x WAnT x Bouts. Furthermore, factorial ANOVA's were the statistical tests used to assess the effect of treatment on body weight, height, and protein intake. If needed, a Tukey's HSD was utilized as the preferred test during any post-hoc analysis. In addition, bivariate correlation and Spearman's rank-order correlation (rho) were performed with PAPr (30-s WAnT) and percent fiber types.

An alpha level of $p < 0.05$ was implemented for all test of significance and post-hoc analyses. Finally, all statistical analyses were performed by the SPSS statistical software package, version 8.0.

CHAPTER 4

RESULTS

Muscle Biopsies and Fiber Type Determination

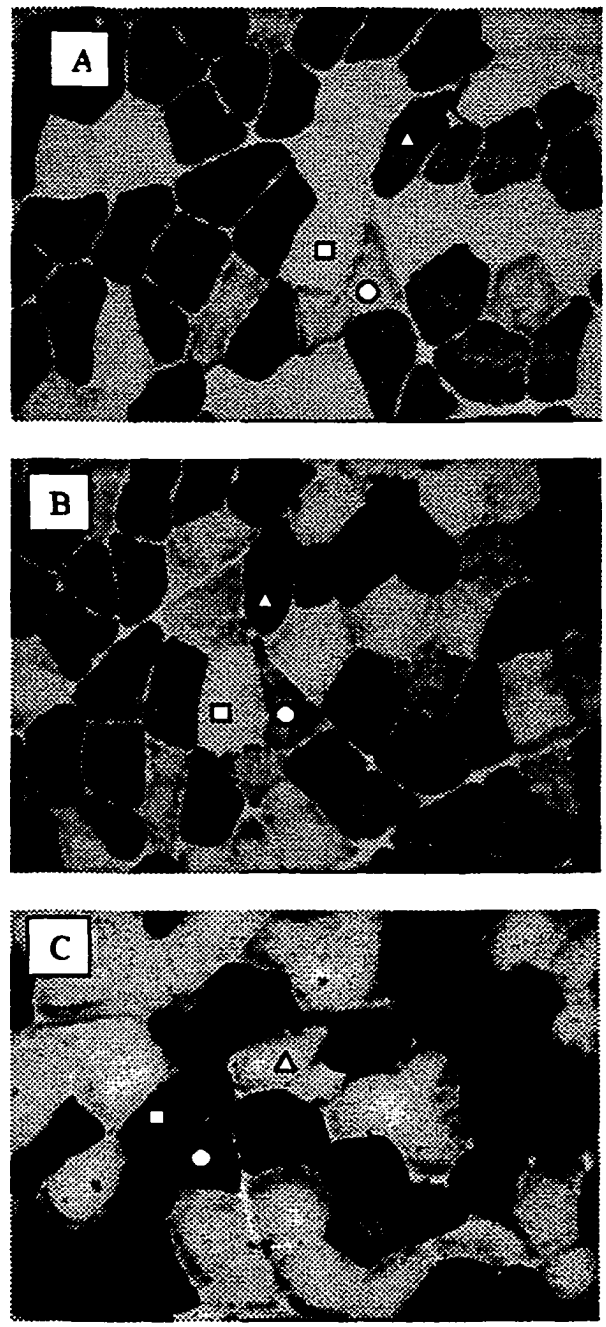
With preincubation pH's of 4.30, 4.54, and 10.15, the myosin-ATPase staining technique (Brooke and Kaiser, 1970) resulted in the greatest differentiation in respect to Type I, IIa, and IIb muscle fibers for our specimens. It is important to demonstrate to the reader that the researchers were able to differentiate fiber types and determine fiber type composition (% Type I, % Type II, % Type IIa, and % Type IIb). Figure 4 represents three serial sections (sections A, B, & C) of muscle from a single participant. Section A (pH of 4.30) reveals that Type I muscle fibers were activated and therefore stained with a dark intensity, whereas Type II muscle fibers were inactivated and light in intensity (Brooke and Kaiser, 1970). The opposite occurs with a preincubation pH of 10.15 (section C), with Type II fibers now being activated (dark intensity) and Type I fibers inactivated (light intensity). At a pH of 4.54 (section B) Type I fibers remain inactive and light in intensity, whereas Type II now differentiate into Type IIa fibers (light intensity) and IIb fibers (grey intensity).

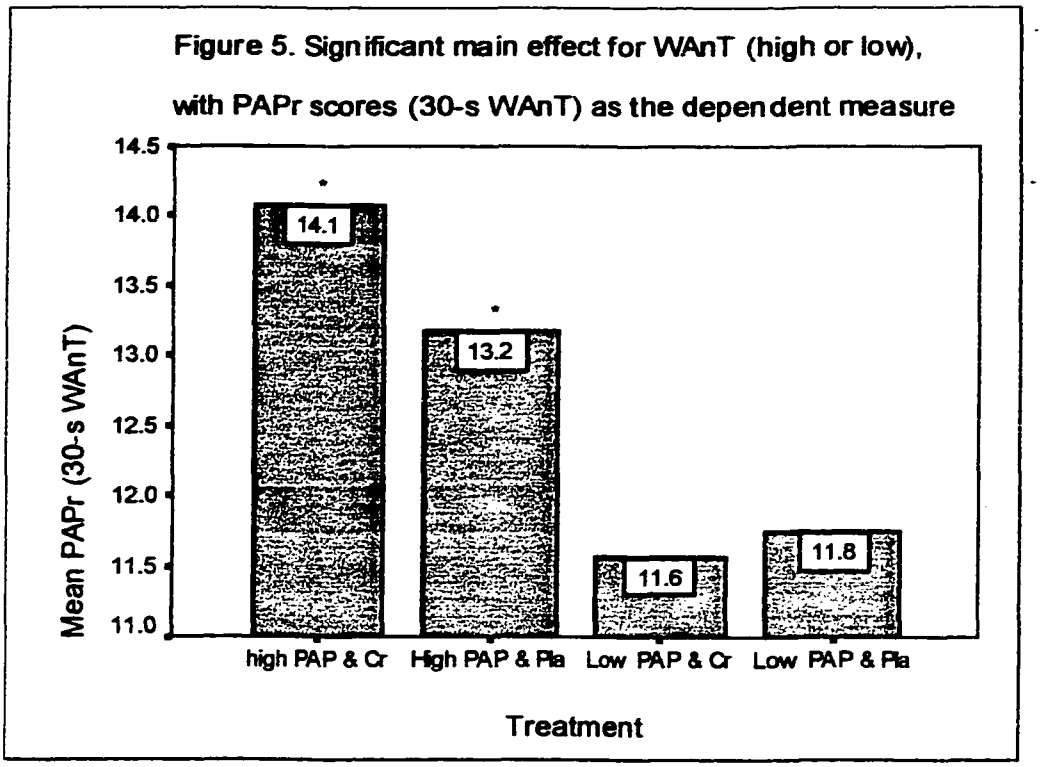
Group Characteristics

Once the groups were formed, it was important that the high and low groups were significantly different in respect to their PAPr scores recorded during the single 30-s WAnT. A 2 (Treatment: creatine or placebo) by 2 (WAnT: high or low) factorial ANOVA, revealed a significant main effect for WAnT ($F(1,15) = 29.14$, $p<0.0001$) as depicted in figure 5, with no other significant main or interaction effects being detected.

This result indicates that our groups will most likely differ significantly as well in respect to percent Type II muscle fibers.

Figure 4- Histochemical stains using the myosin ATPase format with preincubations of (A) pH 4.3, (B) pH 4.54, and (C) pH 10.15 in serial sections of the vastus lateralis muscle group. △ = Type I; □ = Type IIa; ○ =Type IIb.





*significantly different from low PAP with Cr and Pla, $p<0.0001$.

The next step then was to determine if any statistical relationships were evident between PAPr (30-s WAnT) and fiber type composition, specifically percent Type II muscle fibers. A bivariate correlation was first calculated, with PAPr as the predictor and percent Type II muscle fibers as the criterion variable. A positive, but weak correlation was detected between the two variables ($r = 0.52$, $p<0.05$) as depicted in figure 6.

A logical next step was to determine if the ranking of participants according to PAPr (30-s WAnT) and percent Type II muscle fibers were significantly related. This was accomplished by means of a Spearman rank-order correlation (rho) which revealed a positive monotonic relationship ($rho = 0.61$, $p<0.01$) between the same two variables (Table 15).

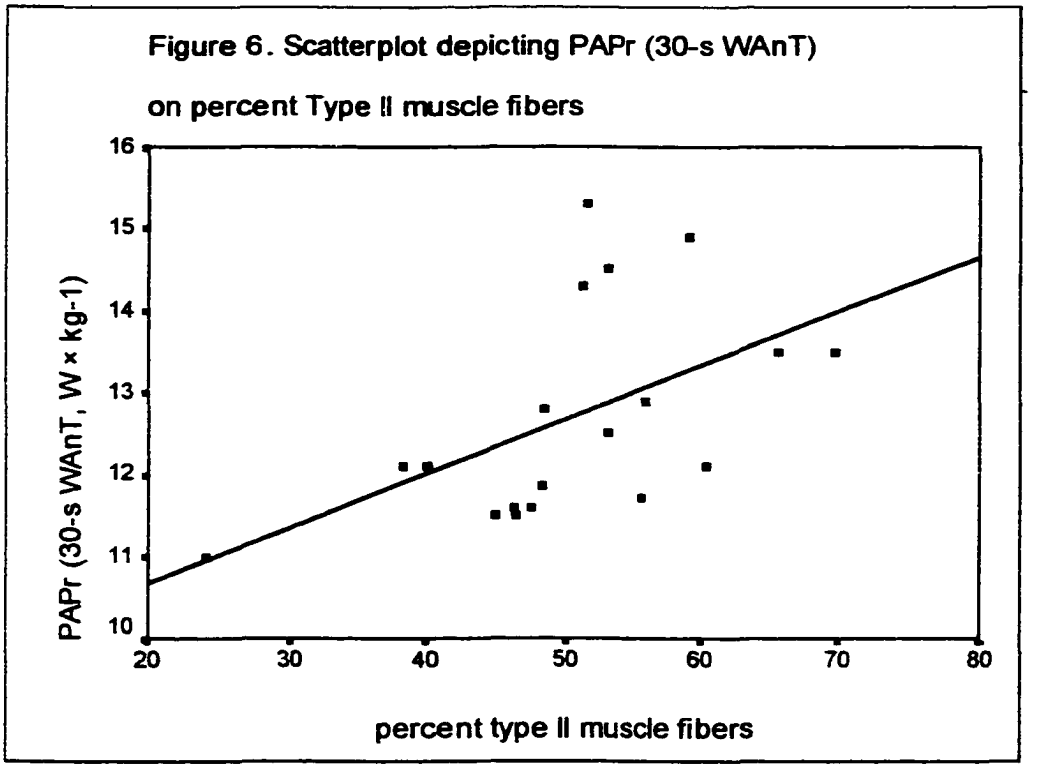
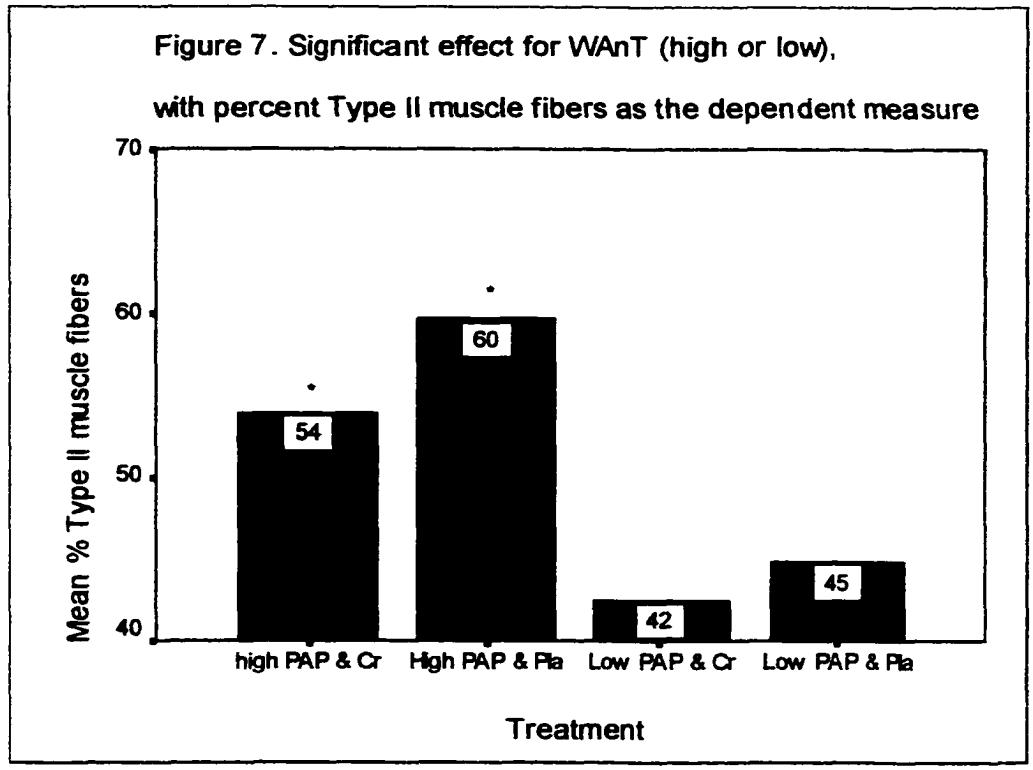


Table 15. Comparison of Rank-Ordering of Participants According to PAPr and percent type II muscle fibers.

Participant	Rank of PAPr	Rank of % Type II	Participant	Rank of PAPr	Rank of % Type II
1	11	3	**11**	11	18
2	1	9	**12**	18	14
3	19	19	**13**	11	17
4	15.5	15	**14**	13	12
5	14	6	**15**	3	7
6	5.5	1	**16**	2	4
7	9	8	**17**	7	5
8	8	11	**18**	17	16
9	4	10	**19**	5.5	2
10	10	15.5			

Finally, a 2 (treatment: creatine or placebo) by 2 (WAnT: high or low) factorial ANOVA, with a dependent measure of percent Type II muscle fibers revealed only a significant main effect again for WAnT (F (1,15) = 12.83, $p<0.01$) as depicted in figure 7. The significant correlation, Spearman's rank-order correlation and the factorial

ANOVA revealed that our "high and low Wingaters" according to PAPr scores obtained during the single 30-s WanT test, were also significantly different in respect to percent Type II muscle fibers.



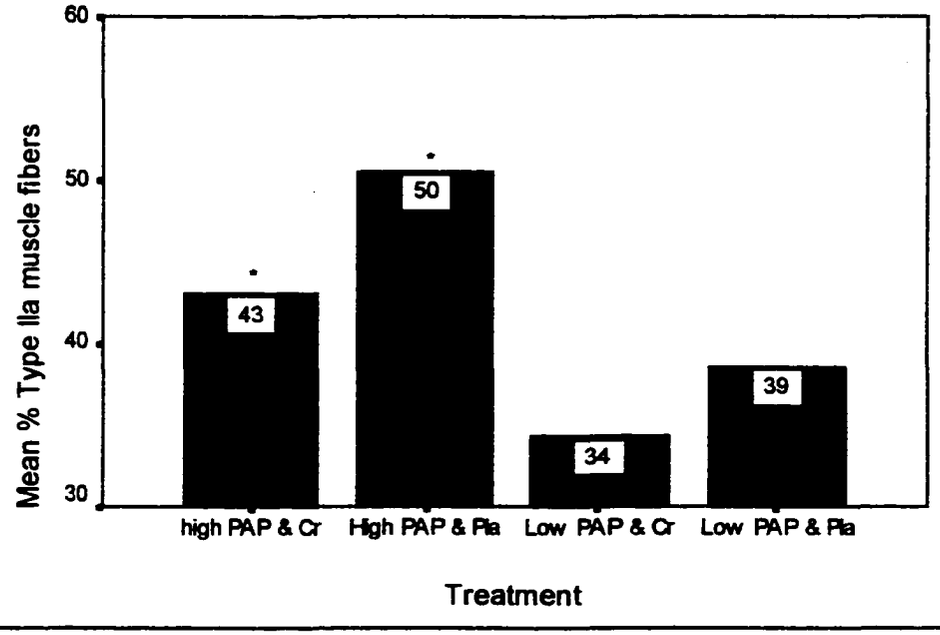
*significantly different from low PAP with Cr and Pla, $p<0.01$.

The same statistical procedures were utilized with percent Type IIa muscle fibers as the dependent measure. A non-significant correlation of $r = 0.43$ ($p > 0.05$) was detected with PAPr scores (predictor) and percent Type IIa muscle fibers (criterion variables). The Spearman's rank order correlation test revealed that the rankings of the participants according to PAPr and percent Type IIa muscle fibers were significantly related ($rho = 0.56$, $p<0.05$). Finally, a 2 (treatment: creatine or placebo) by 2 (WAnT: high or low) factorial ANOVA, with a dependent measure of percent Type IIa muscle

fibers, revealed a significant main effect only for WAnT ($\underline{F}$ (1,15) = 7.42, $p<0.05$) as depicted in figure 8.

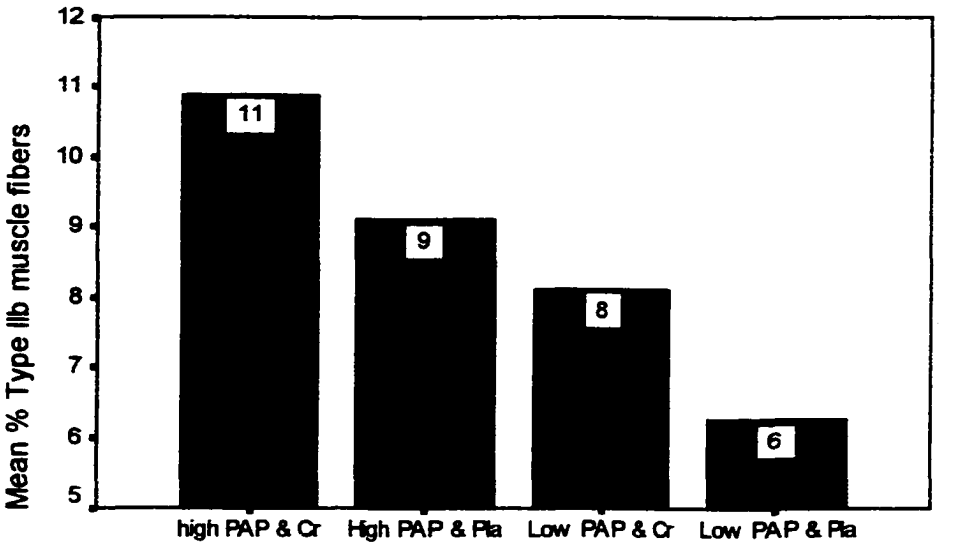
Percent Type IIb muscle fibers was also analyzed as the dependent measure with the aforementioned statistical tests. A non-significant correlation of $r = 0.22$ ($p<0.05$) and a Spearman's rank order correlation of rho = 0.21 ($p<0.05$) was detected between PAPr and percent Type IIb muscle fibers. Furthermore, a 2 (treatment: creatine or placebo) by 2 (WAnT: high or low) factorial ANOVA, with a dependent measure of percent Type IIb muscle fibers, revealed a non-significant main effect again for WAnT ($\underline{F}$ (1,15) = 7.42, $p<0.05$) as depicted in figure 9. No other significant main or interaction effects were detected.

Figure 8. Significant main effect for WAnT (high or low), with percent Type IIa muscle fibers as the dependent measure



*significantly different from low PAP with Cr and Pla, $p<0.05$.

Figure 9. Nonsignificant effect for WAnT (high or low), with percent Type IIb muscle fibers as the dependent measure



Anthropometric and Nutritional Analysis Data

Table 16 displays the physical characteristics, age, and protein intake and urinary volume at pre and post-supplementation. Creatine supplementation has been reported to significantly increase body mass and therefore a 2 (Time: pre or post) by 2 (WAnT: high or low) by 2 (Treatment: creatine or placebo) split-plot Factorial ANOVA, with body mass as the dependent measure was performed. Only a significant main effect for Time ($F(1,15) = 7.1$, $p<0.05$) was detected (Appendix G).

As mentioned within the methodology, pre-supplementation and post-supplementation nutritional assessments were used to control for protein intake. These data were analyzed once again by a 2 (Time: pre or post) by 2 (WAnT: high or low) by 2 (Treatment: creatine or placebo) split-plot Factorial ANOVA, with protein intake as the dependent measure. No significant main or interaction effects were found (Appendix G).

Table 16. Anthropometric and protein intake data for pre and post-supplementation.

	Age (yrs)	Height (cm)	Body Mass (kg)		Protein Intake (gr.)	
			Pre	Post	Pre	Post
Creatine	**21.30**	**178.67**	**82.97**	**83.83**	**95.77**	**94.67**
n = 10	± 1.5	*±8.1*	*±16.5*	*±16.5*	*±12.7*	*±13.2*
Placebo	**22.20**	**180.59**	**85.34**	**85.72**	**99.44**	**96.38**
n = 9	±2.2	*±7.6*	*±11.6*	±12.3	*±19.4*	*±24.6*
Gr. # 1	**21.60**	**177.80**	**78.94**	**79.90**	**98.48**	**96.24**
n = 5	*±0.9*	*±3.7*	*±13.5*	±13.7	*±13.8*	*±13.0*
Gr. # 2	**23.20**	**184.35**	**82.82**	**83.00**	**95.06**	**89.72**
n = 5	*±2.6*	*±2.5*	*±2.4*	*±2.4*	*±18.8*	*±29.6*
Gr. # 3	**21.00**	**179.54**	**87.00**	**87.76**	**93.04**	**93.10**
n = 5	*±2.0*	*±11.4*	*±19.8*	*±19.7*	*±12.3*	*±14.7*
Gr. # 4	**21.00**	**175.87**	**88.50**	**89.13**	**104.91**	**104.70**
n = 4	*±0.8*	*±10.1*	*±18.2*	*±19.1*	*±21.5*	*±16.7*

Gr. #1 = creatine and high WAnT, Gr. # 2 = placebo and high WAnT, Gr. # 3 = creatine and low WAnT, and Gr. # 4 = placebo and low WAnT, NA = not applicable.

Repeated Bouts of the WAnT

Tables 17-20 displays power measurements recorded during each of the 5 repeated bouts of the WAnT. Each table represents one group and allows for comparison of performance at pre and post-supplementation. Statistical output is provided for each of the five dependent variables, expressed as change scores in Appendix G. There were no significant main or interaction effects ($p > 0.05$) for any of the five dependent measures when analyzed by means of 2 (Treatment: creatine or placebo) by 2 (WAnT: high or low) by 5 (Bouts: 1 to 5) factorial ANOVA's.

Table 17 Performance data for 5 repeated bouts of the WAnT (Group #1).

			PAPr (Watts/kg)		PAPa (Watts)		MAPr (Watts/kg)		MAPa (Watts)		FI (Watts)	
			X	SD (±)	X	SD (±)	X	SD (±)	X	SD (±)	X	SD (±)
High PAPr + Creatine (Group #1)	**Bout #1**	**Pre**	10.8	0.4	855	165.0	9.6	0.3	757	120.9	21.8	8.0
		Post	**11.0**	**0.3**	**880**	**155.4**	**9.6**	**0.7**	**760**	**108.2**	**26.8**	**11.4**
	Bout #2	**Pre**	9.5	0.4	749	122.9	7.9	0.3	625	102.1	30.0	3.7
		Post	**9.7**	**0.5**	**778**	**158.6**	**7.9**	**0.3**	**637**	**118.6**	**33.2**	**4.7**
	Bout #3	**Pre**	8.1	0.7	638	119.5	6.6	0.5	525	88.3	30.8	3.7
		Post	**8.3**	**0.7**	**664**	**146.3**	**6.6**	**0.5**	**531**	**119.4**	**35.6**	**4.0**
	Bout #4	**Pre**	6.9	0.9	548	141.3	5.6	0.5	450	108.9	30.8	8.1
		Post	**7.4**	**0.8**	**597**	**142.4**	**5.8**	**0.5**	**469**	**111.0**	**37.4**	**5.0**
	Bout #5	**Pre**	6.6	0.9	523	137.6	5.3	0.6	424	105.1	32.2	6.7
		Post	**6.9**	**1.0**	**558**	**149.3**	**5.5**	**0.6**	**445**	**117.4**	**36.8**	**8.8**

Table 18. Performance data for 5 repeated bouts of the WAnT (Group #2).

			PAPr (Watts/kg)		PAPa (Watts)		MAPr (Watts/kg)		MAPa (Watts)		FI (Watts)	
			X	SD (±)	X	SD (±)	X	SD (±)	X	SD (±)	X	SD (±)
High PAPr + Placebo (Group #2)	**Bout #1**	Pre	10.6	0.6	883	67.0	9.5	0.2	787	23.3	19.0	8.2
		Post	**10.7**	**0.4**	**889**	**37.4**	**9.5**	**0.3**	**789**	**35.7**	**21.0**	**3.4**
	Bout #2	Pre	9.7	0.6	801	42.7	8.1	0.4	654	62.1	28.8	3.2
		Post	**9.6**	**0.3**	**795**	**19.6**	**8.1**	**0.5**	**671**	**47.7**	**28.6**	**6.8**
	Bout #3	Pre	8.6	0.9	716	79.4	6.9	0.8	578	64.6	34.0	6.8
		Post	**8.67**	**1.0**	**718**	**79.4**	**7.0**	**0.9**	**581**	**74.0**	**34.0**	**3.2**
	Bout #4	Pre	7.9	1.3	659	99.7	6.3	0.9	522	76.3	36.0	3.2
		Post	**7.9**	**1.0**	**661**	**81.7**	**6.4**	**1.0**	**532**	**77.4**	**35.0**	**2.7**
	Bout #5	Pre	7.6	1.3	624	112.5	6.1	1.0	503	87.8	33.5	5.7
		Post	**7.8**	**1.1**	**648**	**89.7**	**6.1**	**0.9**	**510**	**78.2**	**38.0**	**5.1**

Table 19. Performance data for 5 repeated bouts of the WAnT (Group #3).

			PAPr (Watts/kg)		PAPa (Watts)		MAPr (Watts/kg)		MAPa (Watts)		FI (Watts)	
			X	SD (±)	X	SD (±)	X	SD (±)	X	SD (±)	X	SD (±)
Low PAPr + Creatine (Group #3)	**Bout #1**	Pre	10.3	0.5	890	206.2	9.2	0.6	790	149.3	20.0	8.2
		Post	**10.2**	**0.6**	**893**	**187.1**	**9.1**	**0.6**	**787**	**144.1**	**23.2**	**7.3**
	Bout #2	Pre	9.3	0.8	801	149.4	7.8	0.7	673	117.3	29.6	5.0
		Post	**9.6**	**0.5**	**833**	**158.5**	**7.8**	**0.7**	**672**	**123.1**	**34.2**	**6.6**
	Bout #3	Pre	8.7	0.5	745	145.6	6.7	0.5	583	116.6	38.4	6.4
		Post	**8.3**	**0.5**	**722**	**127.2**	**6.6**	**0.7**	**571**	**96.4**	**37.0**	**7.1**
	Bout #4	Pre	7.6	0.4	667	171.6	5.8	0.3	508	112.9	41.0	5.2
		Post	**7.4**	**0.6**	**645**	**126.4**	**5.8**	**0.5**	**505**	**96.3**	**39.2**	**4.9**
	Bout #5	Pre	6.9	0.3	604	149.1	5.5	0.4	472	92.8	37.6	4.9
		Post	**7.1**	**0.5**	**617**	**130.2**	**5.5**	**0.4**	**478**	**95.1**	**39.6**	**4.6**

Table 20. Performance data for 5 repeated bouts of the WAnT (Group #4).

			PAPr (Watts/kg)		PAPa (Watts)		MAPr (Watts/kg)		MAPa (Watts)		FI (Watts)	
			X	SD (±)	X	SD (±)	X	SD (±)	X	SD (±)	X	SD (±)
Low PAPr + Placebo (Group #4)	**Bout #1**	Pre	10.6	0.6	949	237.4	9.5	0.5	848	219.2	20.8	3.5
		Post	**10.4**	**1.2**	**919**	**183.8**	**8.9**	**0.7**	**789**	**145.6**	**25.5**	**6.8**
	Bout #2	Pre	9.4	0.7	795	123.9	7.9	0.4	705	176.2	28.8	8.8
		Post	**9.3**	**0.7**	**823**	**144.8**	**7.5**	**0.8**	**656**	**95.6**	**36.0**	**8.3**
	Bout #3	Pre	8.4	0.6	711	130.5	6.9	0.4	611	153.4	33.5	7.0
		Post	**8.1**	**0.4**	**722**	**154.7**	**6.5**	**0.8**	**575**	**94.8**	**34.3**	**12.5**
	Bout #4	Pre	7.7	0.6	638	143.9	6.1	0.2	545	122.9	38.5	6.9
		Post	**7.6**	**0.3**	**678**	**133.7**	**5.9**	**0.7**	**517**	**77.5**	**40.5**	**12.9**
	Bout #5	Pre	7.2	0.7	595	138.6	5.7	0.2	506	97.67	38.5	11.4
		Post	**7.4**	**0.6**	**660**	**155.4**	**5.7**	**0.7**	**508**	**83.0**	**40.0**	**15.7**

CHAPTER 5

DISCUSSION

Group Characteristics

It is important to remember that originally sixty-five participants performed a single 30-s WAnT, and PAPr scores were rank-ordered from highest to lowest. Since the ten participants with the highest and lowest PAPr scores did not all volunteer to partake in the remainder of the study, it was essential to test to see if the "high and low" WAnT groups were in fact significantly different in respect to PAPr scores obtained during the single 30-s WAnT. It was apparent that the 10 participants within the high group had higher PAPr scores, regardless of treatment (figure 5). As previously mentioned within the methodology, an essential assumption of this study was that participants with higher PAPr scores obtained during the single 30-s WAnT would possess higher percentages of Type II muscle fibers (Bar-Or et al., 1980; Inbar, Kaiser, and Tesch, 1981; Kaczcowski et al., 1982; Froese and Houston, 1987). In other words, groups were formed according to exercise performance in the hopes that the high WAnT groups would also possess a higher percentage of Type II muscle fibers than the low WAnT groups.

One of the key findings of this study was the positive relationship between muscle fiber type composition, when expressed as percent Type II muscle fibers and PAPr ($W \cdot kg^{-1}$) during the single WAnT. This conclusion was based on the following information. Firstly, bivariate correlation analysis revealed that percent Type II muscle fibers (predictor) and PAPr (criterion) were significantly correlated (figure 6). However, the small r of 0.52 reveals that the predictor variable was only capable of explaining 27% ($r^2 = 0.27$) of the variance seen in the criterion variable. Furthermore, a Spearman's rank-order

correlation was used to see if the participants would hold their ranks. It was expected that those participants with high rankings according to PAPr (predictor) would also have correspondingly high rankings for percent Type II muscle fibers (criterion). The positive monotonic relationship ($R = 0.611$, $p<0.01$) between the variables revealed that there was a significant relationship between the ranking of participants according to the two variables. The data from this study demonstrates a statistically significant, but weak relationship in the ranking of participants according to PAPr and percent Type II muscle fibers. More specifically, not all participants with high PAPr scores possessed high percentages of Type II muscle fibers. Finally, since the participants actual fiber composition was calculated (% Type I, % Type II, % Type IIa and % Type IIb) the researcher could test to see if the groups were also significantly different in this respect. A 2 x 2 factorial ANOVA revealed a significant main effect for WAnT, in which the high PAPr group had significantly higher values for percent Type II muscle fibers, regardless of treatment (figure 7). These findings were essential to the study design as they revealed that although participants were grouped according to PAPr scores obtained during the single 30-s WAnT, the groups also differed in fiber type composition, when expressed as percent Type II muscle fibers.

As stated in the results section, the same statistical procedures were performed with the percent Type IIa and IIb muscle fibers as the dependent measure. Although a significant bivariate regression and factorial ANOVA were detected with percent Type IIa muscle fibers, the study focused on Type II muscle fibers since all three statistical analyses were significant and to a greater degree. No significant relationships were detected with percent Type IIb as the dependent measure. For the purpose of this study however,

participants with higher PAPr scores obtained during the single 30-s WAnT possessed higher percentages of type II muscle fibers.

Repeated Bouts of the WAnT

The major finding of this study, as related to the statement of the problem, was that 5 days of creatine supplementation (4 x 20g/day) had no detectable effect on 5 repeated bouts of the WAnT (15-s in duration against a resistance of 0.075 kg, with 45-s rest periods), regardless of how participants were classified for the 30-s WAnT (high or low PAPr). This finding was unexpected since most research involving repeated bouts of high intensity exercise support creatine supplementation as having an ergogenic effect (Balsom et al., 1993 & 1995; Birch et al., 1994; Casey, et al., 1996; Dawson et al., 1995; Earnest et al., 1995; Greenhaff et al., 1993 & 1994; Kirksey et al., 1997; Lemon et al., 1995; Schneider et al., 1997; Vandenberghe et al. (as cited in Williams and Brach, 1998); Volek et al., 1997; Ziegenfuss, Lemon, Rogers, Ross, and Yarasheski, 1997). In fact, Williams and Branch (1998) reviewed 17 articles related to creatine supplementation and cycle ergometry, and stated that eleven of those articles reported creatine as being beneficial to performance (25% ± 29% improvement) and only five failing to report creatine has being beneficial to performance.

It was expected that participants with high and low WAnT scores, and supplemented with placebo (groups #2 and # 4 respectively) would have no significant change in performance from pre to post-supplementation. It was also expected that those with high and low WAnT scores who received creatine (groups #1 and #3 respectively) would show an improvement in performance, with the greatest effect seen in group #1

(high WAnT and creatine). The hypothesis that high PAPr scores and creatine supplementation would result in the greatest improvement in performance was based on the following. Firstly, it has been proposed that higher intramuscular creatine stores might facilitate phosphocreatine resynthesis during the recovery phase of repeated bouts of high intensity exercise (Greenhaff et al., 1994). Secondly, phosphocreatine has been shown to be an effective buffer for H+ and would restrict the formation of adenosinediphosphate, the starting point for adenine nucleotide degradation (Greenhaff 1999 et al., 1993). In addition (Tesch et al., 1989; Soderlund et al., 1992) have reported that Type II muscle fibers store and utilize significantly more phosphocreatine during intermittent, high intensity exercise than do Type I muscle fibers and therefore, Type II muscle fibers may also *uptake or retain* more of the supplemental creatine than Type I muscle fibers. Finally, it is important to recall that the high and low groups, according to PAPr scores obtained during the single 30-s WAnT were significantly different in respect to fiber type composition (percent Type II muscle fibers).

Based on the information present within the previous paragraph, it was hypothesized that Type II muscle fibers would uptake more of the supplemental creatine, resulting in greater ATP and phosphocreatine resynthesis. Therefore group #1, with a higher a percentage of Type II muscle fibers and receiving creatine supplementation, would experience the greatest uptake of creatine with a consequent improvement in performance. To address this question, it would be necessary to obtain pre and post-supplementation muscle samples, determine fiber type distribution, and analyze for creatine, phosphocreatine, and adenosinetriphosphate levels. However in this study, we were restricted to a determination of fiber type distribution only, but a review of previous

studies reveal that creatine supplementation should have raised total creatine levels within muscle (Greenhaff et al., 1994; Harris et al., 1992; Hultman et al., 1996). As previously reported, no significant main or interaction effects were detected for any of the dependent measures across all five bouts of the WAnT (Appendix G). Theoretically, creatine supplementation could be effective after numerous bouts of high intensity exercise. This prediction was based on the work of Balsom et al (1995) who had participants perform five bouts of maximal cycling at 140 revolutions/minute, against a resistance that produced maximum work and found that phosphocreatine levels were only significantly higher for the treatment group after the fifth bout of high intensity cycle performance. Consequently, one would expect performance only to improve after creatine supplementation has an effect at the metabolic level (i.e. increased levels of phosphocreatine).

An improvement in performance for PAP and MAP (absolute and relative) would be indicated by negative change scores (pre minus post) and would represent greater scores being achieved after five days of creatine supplementation. It was expected that group #1, with a higher percentage of Type II muscle fibers and having received creatine supplementation, would have significantly greater, negative change score values (pre minus post) for PAPa, PAPr, MAPa, and MAPr than the other three groups. If this prediction had been true, a graph similar to figure 10 might have resulted. In reviewing figure 10, one could expect the placebo groups (#2 and #4) to show no improvement in performance and have lines that are basically above the "0.0" mark on the ordinate or show no consistent pattern of negative change scores. The treatment groups however (#1 and #3), might have lines that begin around the "0.0" mark on the ordinate, but begin to

steadily slope downwards towards bout # 5, with ever increasing negative change scores. Figures 11-14 show the actual outcomes for the four groups in regards to change scores for PAP and MAP (relative and absolute). As predicted, the placebo groups showed no improvement in performance. However, the creatine groups also showed no signs of improvement in performance as the lines undulated in an unpredictable manner.

Figure 10. Line graph depicting expected outcome of treatment on PAP & MAP (absolute & relative) during 5 bouts of the WAnT

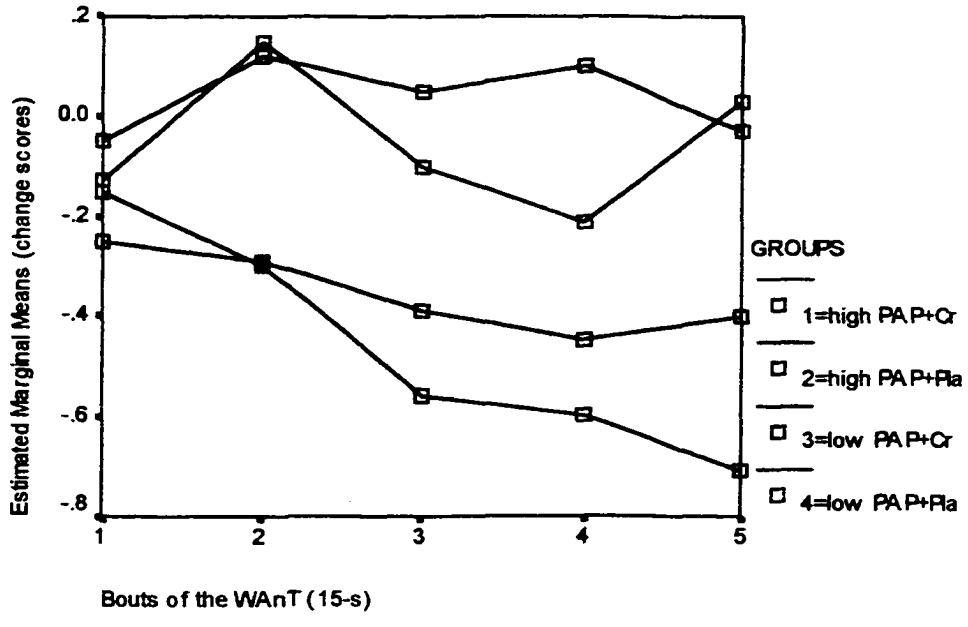


Figure 11. Line graph depicting nonsignficant effect of treatment on PAPa (change scores) during 5 bouts of the WAnT

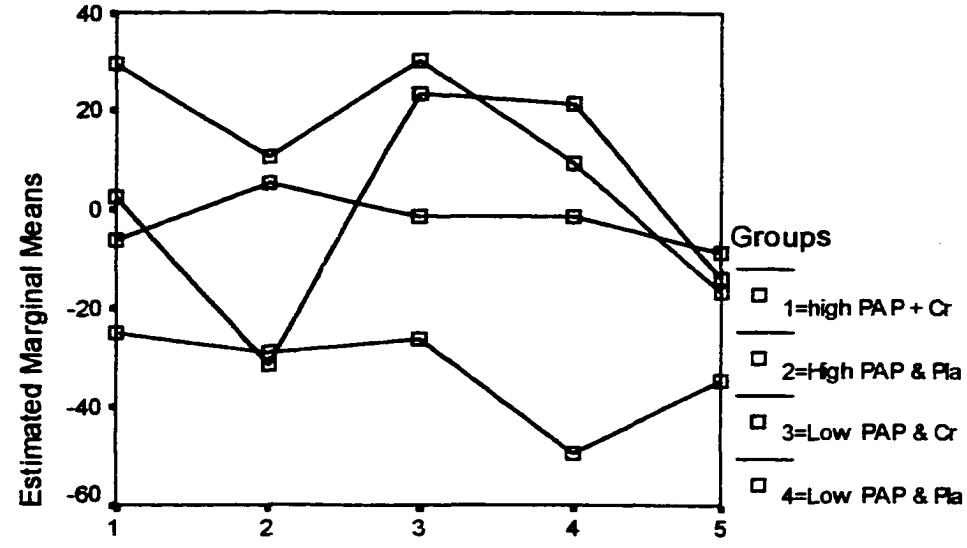


Figure 12. Line graph depicting nonsignifican effect of treatment on PAPr(change scores) during 5 bouts of the WAnT

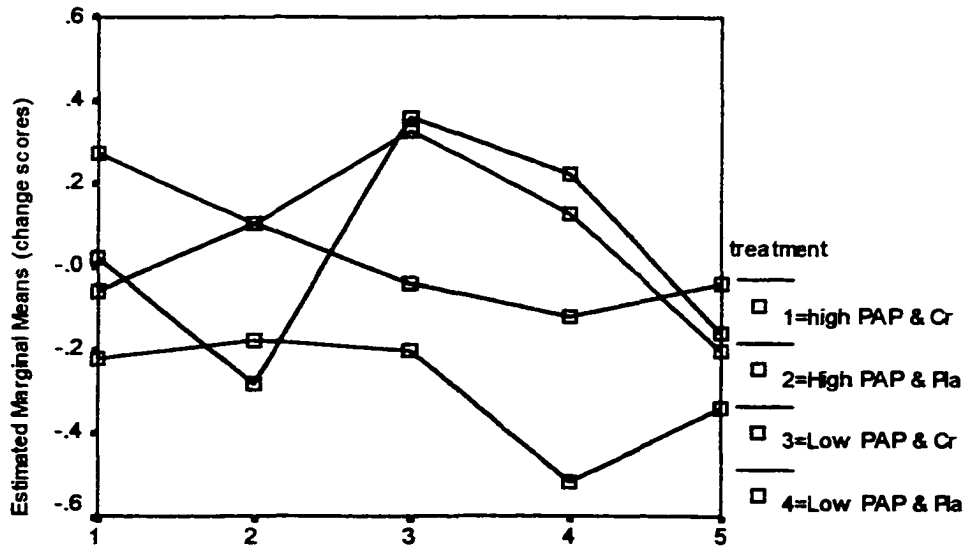


Figure 13. Line graph depicting nonsignificant effect of treatment on MAPa (change scores) during 5 bouts of the WAnT

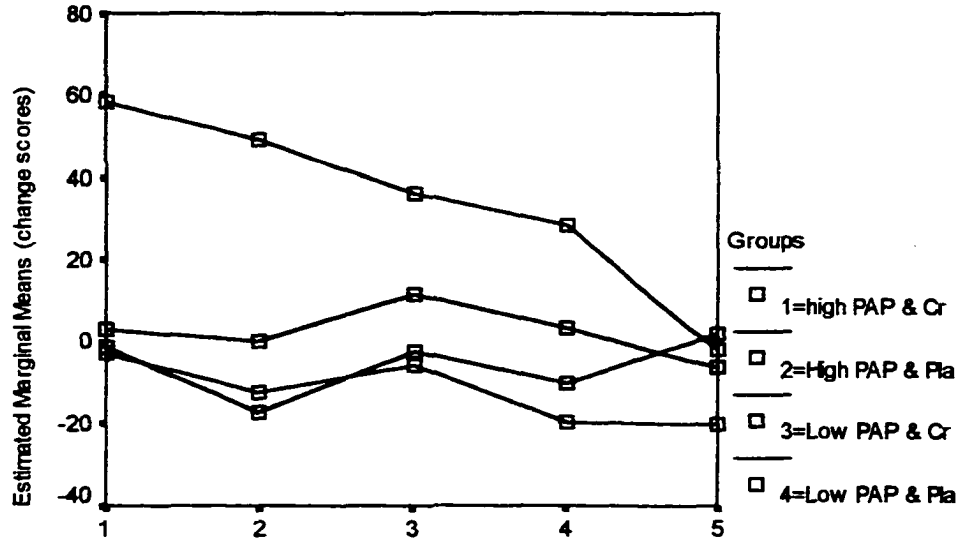
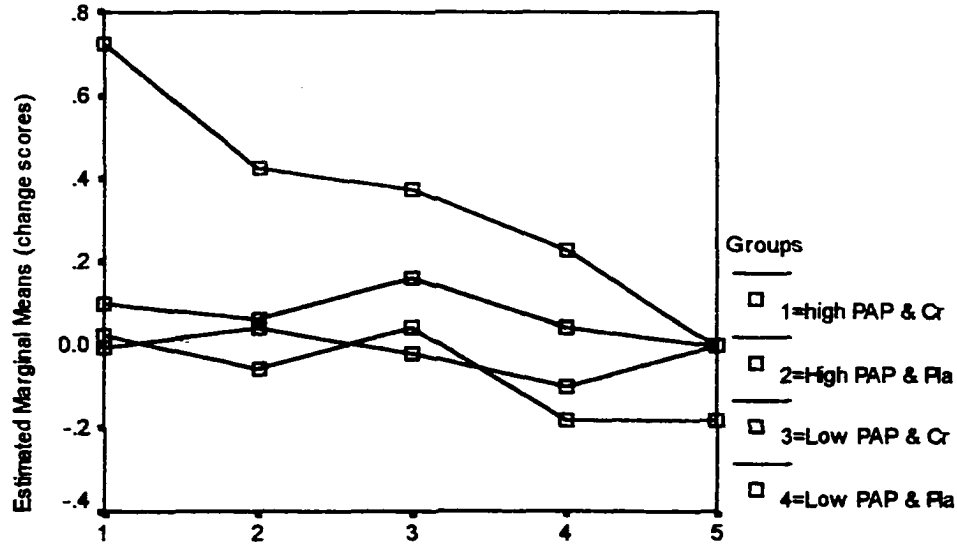
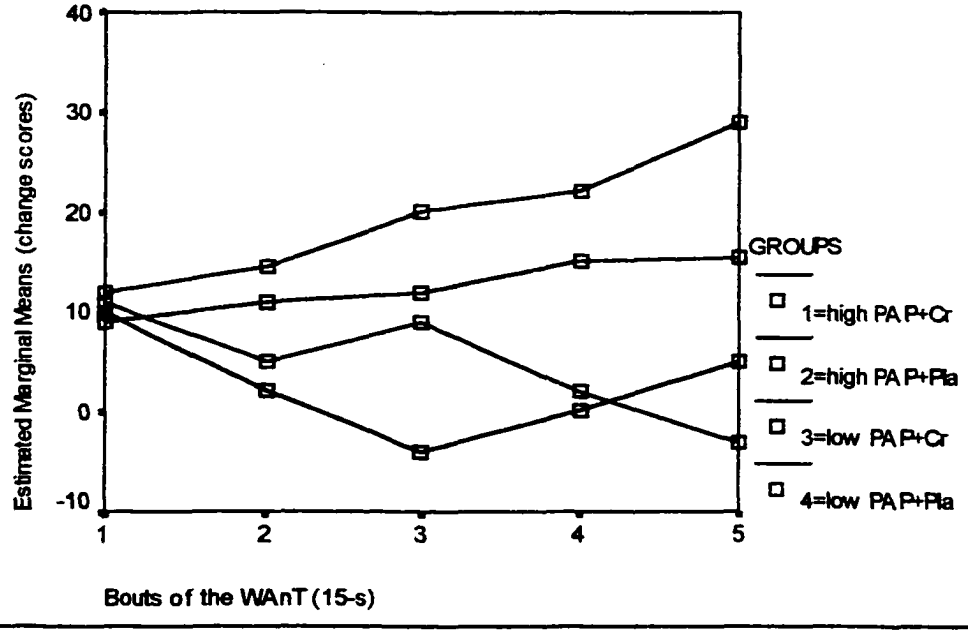


Figure 14. Line graph depicting nonsignificant effect of treatment on MAPr (change scores) during 5 bouts of the WAnT



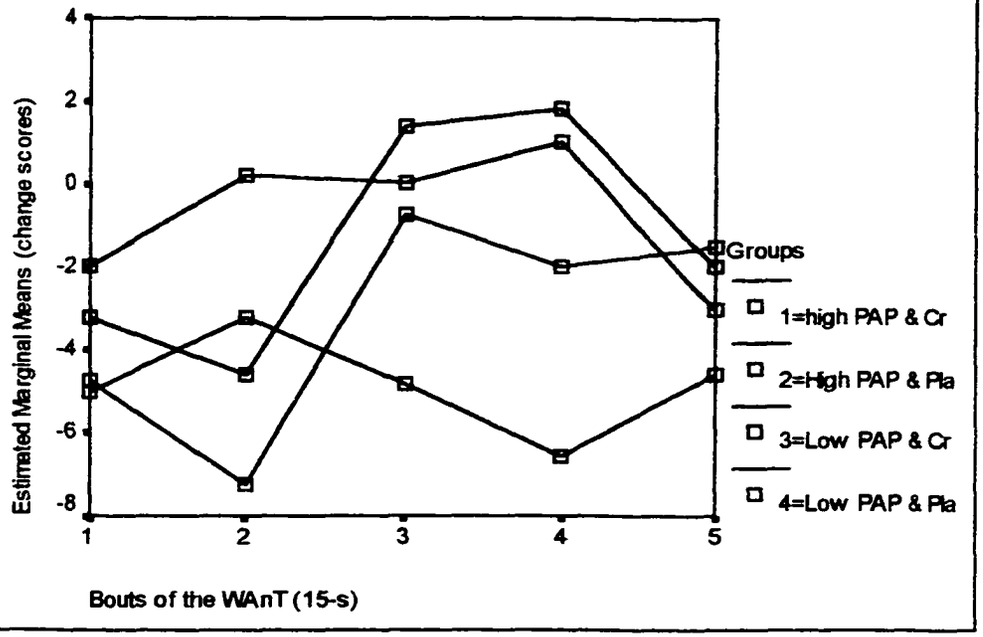
The last dependent measure of interest was the fatigue index (FI), which examined the degree of power drop-off during the WAnT (Inbar, Bar-Or and Skinner, 1996). Based on the information within the first paragraph on page 55, it was also anticipated that group #1, with a higher percentage of Type II muscle fibers and supplemented with creatine, would experience a greater ability to maintain power output during the WAnT (Balsom et al., 1995). Improvements in performance for FI would be indicated by positive, rather than the negative change scores as previously mentioned for MAP and PAP. Therefore, change scores for FI (pre minus post) would be significantly greater for group #1 than the other three groups and again, it was hypothesized that only after several bouts of high intensity exercise would the benefits of creatine supplementation be detected (figure 15).

Figure 15. Line graph depicting expected outcome of treatment on FI during 5 bouts of the WAnT



However, the actual outcome again was not similar to the expected outcome as evidenced figure 16. All four groups produced lines that were random in nature and displayed no maintenance of power across all five bouts (figure 16).

Figure 16. Line graph depicting nonsignificant effect of treatment on FI (change scores) during 5 bouts of the WAnT



The results obtained in this study are in agreement with only a few of the studies that have investigated creatine supplementation on repeated bouts of high intensity exercise. Barnett, Hinds, and Jenkins (1996) reported that 20g of creatine/day for 4 days had no effect on peak power or mean power output (absolute or relative) during repeated sprint cycle performance (7 x 10 bouts with 30-s of passive rest between bouts 1-5, and 5-min between bouts 5-7), with a gear ratio eliciting 8.87 rev/pedal crank revolution. These researchers concluded that either creatine supplementation failed to significantly raise muscle creatine concentration or creatine concentration was significantly increased,

but performance during 7 x 10-s cycle sprints was not affect. Moreover, Gonzalez de Suso et al. (as cited in Williams and Branch, 1998) used a randomized double-blind, placebo-control, cross-over design to examine creatine supplementation (21 g/day for 14 days) on 7 x 7-s bouts of cycle sprinting. The [PCr]/[β-ATP] ratio increased in both groups, but performance results were equivocal, with a 7% increase in peak power detected only when placebo was taken before creatine. The length of the washout period was not provided and consequently the authors felt the time period may have been insufficient and that a treatment effect could have been overlooked (Williams and Branch, 1998). Thirdly, McKenna, Morton, Selig, and Snow (1999) revealed that six doses of 5g of creatine plus 5g of dextrose powder for 5 days significantly raised resting concentrations of creatine, phosphocreatine, and total creatine (creatine + phosphocreatine), but had no effect on five bouts of intermittent maximal cycling. The authors suggested that a placebo effect was responsible for masking the true treatment effect of creatine supplementation. Febbraio et al. (1995) and Redondo et al. (1996) also reported that creatine supplementation was ineffective as an ergogenic aid for repeated bouts of intermittent maximal exercise. However, the length and number of bouts used in their studies were not comparable to the present investigation.

In this study, there are several reasons why creatine supplementation did not produce an ergogenic effect. First, it has been well established that ingestion of approximately 5g of creatine in solution will raise plasma creatine concentration from ~40 μmol/l to 600-800 μmol/l within 1 hour (Green et al., 1996; Harris et al., 1992). Furthermore, ingesting 20 g/day for 5 days can increase intramuscular creatine levels by 20%, of which 20% can be found as phosphocreatine (Greenhaff et al., 1994; Harris et al.,

1992). Based on previous work (Greenhaff et al., 1994; Harris et al., 1992; Hultman et al., 1996) we would assume that our creatine supplementation protocol (4 x 5g of creatine + 2 g of dextrose powder/day for 5 days) would have resulted in similar intramuscular phosphagen levels, but unfortunately muscle total creatine levels (creatine + phosphocreatine) were not measured. However, the possibility exists that our supplementation protocol did not elevate resting intramuscular phosphagen levels to the point needed to meet the demands of five repeated bouts of the WAnT, and consequently, no improvement in performance would be expected. This is highly unlikely, since a majority of research involving creatine supplementation with repeated bouts of intermittent, high-intensity exercise, have reported an ergogenic effect (Williams and Branch, 1998).

Having participants perform 5 x 15-s repeated bouts of WAnT, with 45-s of active rest between bouts, resulted in a work to rest ratio of 1:3. With a half recovery time of phospocreatine stores at 30-s (McArdle, Katch and Katch, 1999) our participants had sufficient time to restore at least 50% of their phosphocreatine levels in preparation for the next bout of exercise. As the bouts progress however, and these participants not being highly trained, the formation of lactate within the muscle might have caused fatigue and account somewhat for the performance results. However (Christensen, Hedman, and Saltin, 1960) have shown that with 15-s work and only 30-s rest (1:2 work to rest ratio), lactate levels were only at 1.8 mmol. It is also possible that 5 bouts of the WAnT did not produce a true "state of fatigue" and that additional bouts might have resulted in our expected outcome. That is group # 1, high PAPr with creatine supplementation would have experienced the greatest improvement in performance as the bouts progressed.

However, our participants were not highly trained and most would have been unable to perform even one additional bout.

Another possible explanation for our unexpected outcomes could be due to the sample sizes per group (i.e. per cell). Although an overall sample size of nineteen was similar with previous studies, the research design employed, a 2 (Time: pre and post) by 2 (Treatment: creatine or placebo) by 2 (WAnT: low or high) by 5 (Bouts: 1 to 5) split-plot factorial ANOVA, was a limiting factor. Diekhoff (1992) stated that sample sizes per cell (group) should be approximately equal, with approximately 10 -15 cases (i.e. participants) per cell for a factorial ANOVA. The placement of the participants into four groups was necessary if the research question was to be answered, and therefore, small sample sizes per group (n=5 for three groups and n= 4 for one group) resulted. This was significant because in a group of 4-5 participants per cell, a single individual could contribute 20% - 25% of the variance in any of the change scores (dependent measure), in comparison to 6.67% - 10% if sample sizes had been 15 or 10 participants per cell. Change scores from a single participant would yield too much influence per cell mean, which could result in statistical non-significance for the factorial ANOVAs. One participant within the treatment group could have been a "non-responder" to creatine supplementation, and as a result could mask the ergogenic effect that might have been present. This is a distinct possibility, as it has been estimated that nearly 30% of those who undergo creatine supplementation do not experience significant increases in creatine and phosphocreatine levels (Greenhaff, 1997). The reasons for this are unclear, but with small sample sizes per cell, a single "non-responder" within the creatine groups could easily affect the data in such a manner that the treatment would appear to have no ergogenic effect on repeated

bouts of the WAnT. In a similar manner, the placebo groups could have masked the ergogenic effect of creatine supplementation, by a single participant experiencing the common "placebo effect".

A possible situation, although unlikely, was proposed by McKenna et al. (1999) to explain the results of their study in which they proposed that a learning effect could have occurred only for the placebo groups, and a creatine-induced ergogenic effect only within the creatine groups. This again could have negated the ergogenic effect of creatine supplementation, but the likelihood of this occurrence seems negligible.

As previously mentioned, exogenous creatine can be consumed in a supplemental or natural form. Throughout the study design, supplemental sources of creatine were monitored, as were natural sources by means of pre and post-supplementation nutritional analyzes. No significant main or interaction effects were detected.

Finally, a limitation of this study was the reliance on the manufacturer (EAS) providing high quality creatine monohydrate. It should be obvious that if the product was not pure creatine, no ergogenic effect would be detected as no "treatment" would have been administered to the creatine groups. The likelihood of creatine not being high quality is highly unlikely, but reported to cover all possibilities.

Body Mass

Numerous articles have reported increases in body mass after creatine supplementation (Balsom et al., 1993; Dawson et al., 1995; and Earnest et al., 1995; Godly and Yates, 1997; Goldberg and Bechtel, 1997;). It was expected that a significant interaction effect for Time x Treatment x WAnT would have been detected, in which

group # 1 (high PAPr + creatine) would have gained weight after 5 days of supplementation. However, both groups gained a significant amount of weight, from pre to post-supplementation (main effect for Time). Increases in body mass with creatine supplementation are thought to be the result of water retention and greater lean body mass. No measurement of water retention or lean body mass was taken and therefore it is unclear if these variables contributed to the significant increase in body mass. Increases in lean body mass are thought to be greatest in those who have low initial intra-muscular creatine concentrations (Balsom et al., 1993; Delanghe et al., 1989; Harris et al., 1992), but this could not be confirmed since muscle creatine analysis was not performed.

To summarize, 20 g of supplemental creatine for 5 days had no effect on 5 repeated bouts of the WAnT, regardless of participants possessing a high or low percentage of Type II muscle fibers. The most likely explanation for this finding was that the number of participants per group was inadequate, which result in any one participant contributing too much influence towards the data. On a more positive note, we found a positive correlation ($r = 0.52$, $p<0.05$) between PAPr (30-s WAnT) and percent Type II muscle fibers, which corresponds to previous research. Furthermore, we were able to form the groups according to PAPr (30-s WAnT), ensuring that the "high and low Wingaters" were significantly different from each other, and based on our prediction, the groups were also significantly different in respect to percent Type II muscle fibers.

CHAPTER 6

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This study examined the influence of fiber type distribution and creatine supplementation on five repeated bouts of the Wingate anaerobic test (WAnT). Sixty-five participants performed a single 30-s WAnT and were ranked-ordered from highest to lowest, according to relative peak anaerobic power (PAPr). Nineteen participants volunteered in such a manner that ten (n=10) represented high PAPr scores and nine (n=9) low PAPr. This was undertaken with the assumption that PAPr scores obtained during a single 30-s WAnT would be positively related to fiber type distribution, expressed as percent type II muscle fibers. Fortunately, muscle fiber typing revealed a positive, but weak relationship between peak anaerobic scores (relative) obtained during the single 30-s WAnT and percent type II muscle fibers ($r = 0.52$, $p<0.05$). This means that although our groups were based on PAPr scores, the groupings (1. high WAnT with creatine, 2. high WAnT with placebo, 3. low WAnT with creatine, and 4. low WAnT with placebo) also reflected muscle fiber type distribution (% type II). Five repeated bouts of the WAnT were performed, each lasting 15-s, against a resistance of 0.075 kg/kg body weight and with 45-s of active rest between each bout. Supplementation occurred for five consecutive days, with groups # 1 and #3 receiving the treatment (4 x 5g of creatine + 2g of dextrose powder) and groups # 2 and # 4 the placebo (4 x 7g of dextrose powder). Peak anaerobic power (absolute and relative), mean anaerobic power (absolute and relative) and rate of fatigue (fatigue index) were recorded during each bout. Statistical analysis was comprised of a series of 2 x 2 x 2 x 5 split-plot factorial ANOVA's, one for

each of the 5 dependent measures (change scores). Body mass was also tested for the effect of creatine supplementation by means of factorial ANOVA's. All four groups experienced a significant increase in body mass from pre to post-supplementation, regardless of Treatment or WAnT. No significant main or interaction effects were detected with protein intake as the dependent measure.

Conclusions

The following conclusions have been reached from the results and discussion of this investigation:

1. No significant main or interaction effects for change scores on any of the 5 dependent measures, regardless of WAnT (high or low PAPr), treatment (creatine or placebo), and bouts (# 1- # 5) were detected.

2. PAPr scores obtained during the single 30-s WAnT were statistically related to fiber type distribution, when expressed as percent type II and IIa.

3. Body mass increased from pre to post-supplementation, regardless of whether participants were high or low (WAnT) and receiving creatine or placebo (Treatment).

Recommendations

1. It is essential that cell sizes be increased to include approximately 10-15 participants.

2. Creatine and phosphocreatine uptake must be measured with blood and muscle samples.

3. If time and money allow, participants should be grouped based on fiber type distribution, instead of relying on the relationship between PAPr scores obtained during a single 30-s WAnT and percent type II muscle fibers.

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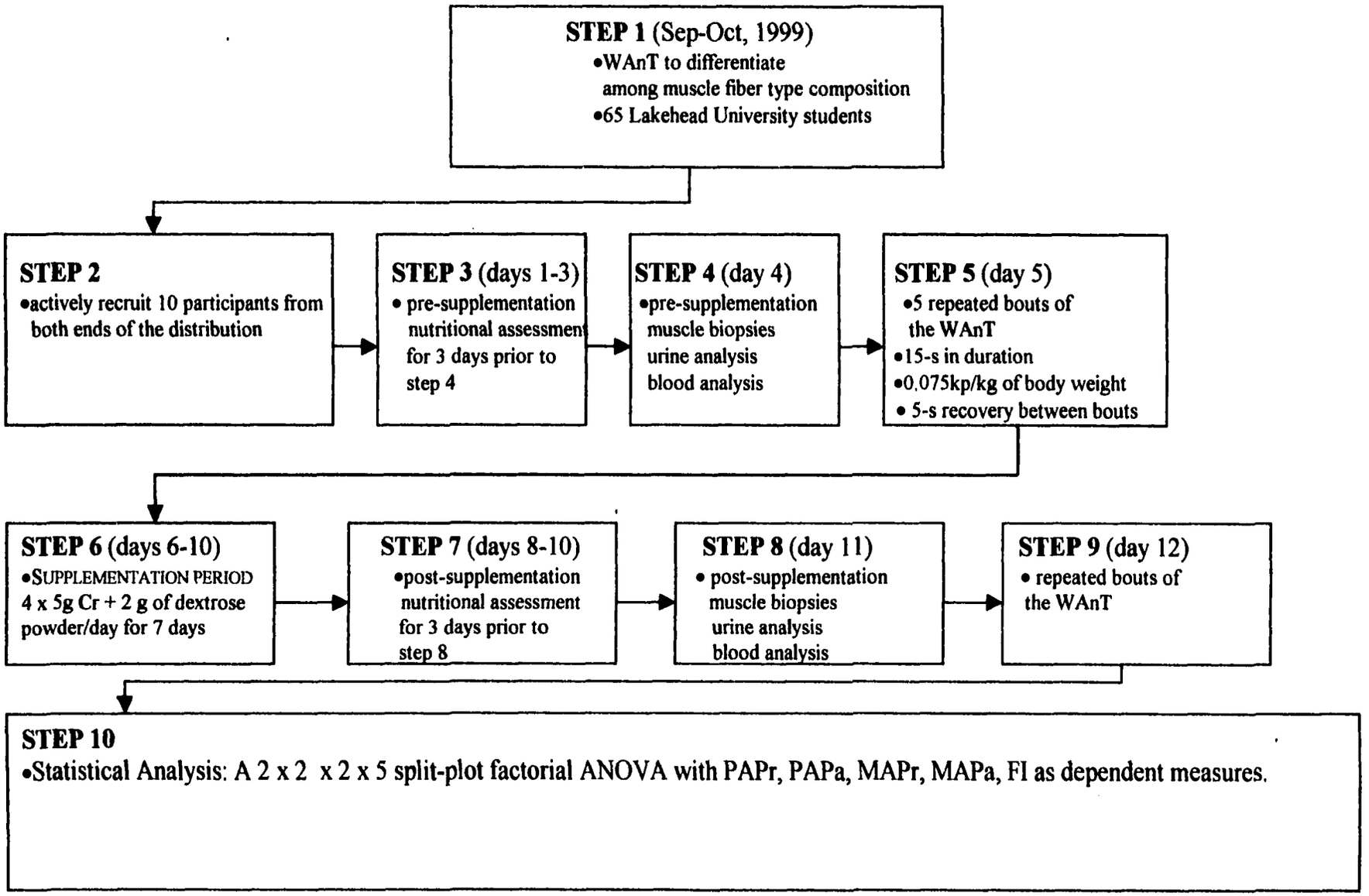
Appendix A

FLOW CHART DEPICTING OUTLINE OF THE METHODOLOGY



Appendix B

"The Influence of Type II Muscle Fibers on the Uptake of Creatine and its Effect on Repeated Bouts of Cycle Ergometry"

October, 1999

SECTION A:

Dear Participants,

I would like to begin by thanking-you for volunteering to be a participant in this research study. Furthermore, feel free to approach me at anytime if you have any questions or concerns related to this research study.

The intent of this research study is to investigate the influence of fiber type distribution on the uptake of supplemental creatine and its effect on repeated bouts of maximal cycle ergometry. More specifically, do those participants with a higher percentage of type II muscle fibers uptake more creatine, and if so, do those same participants demonstrate a greater significant improvement in repeated bouts of cycle ergometry.

A simple and noninvasive means of grouping participants according to muscle fiber type distribution is to record peak anaerobic power (PAP) scores achieved during a single 30 second Wingate test. In other words, high and low PAP scores are correlated with a higher and lower percentage of type II muscle fibers respectively.

The purpose of **SECTION A** in the study is to collect PAP scores from 150-200 undergraduate, male kinesiology students and therefore participants will be asked to perform a single 30 second Wingate test. Following this test, you may experience a brief period of muscular fatigue in your legs and possibly nausea. You will be required to refrain from ingesting food, caffeine, and/or alcohol for three hours prior to the initiation of testing.

From these participants, 40-50 will be asked to volunteer for the study based on their PAP scores and those individuals not approached will have fulfilled their role in this study.

As a volunteer, you have the right to refuse any test and to withdraw from study at any time. All information collected for the purpose of this research will remain confidential through the use of a coding system. No reference to any participants will be made in an any report of the results. The data will be stored by Marc Poirier and by Dr. Norman LaVoie within the Faculty of Kinesiology at Lakehead University for a period of seven

years. The findings of the study will be made available to you at your request upon completion of the project.

You also be required to fill out a modified Physical Activity Readiness Questionnaire (PAR-Q) with one additional question related to local anesthetics. If as a subject you answer **yes** to any of the questions on the modified PAR-Q, your participation in this study will not be needed.

If you have any questions concerning the study, I may be reached by phone at home (807)-577-0304 or at work (807)-346-7815. I may also be reached by means of email at *(mpoirier@ice.lakeheadu.ca).*

Marc Poirier
Principal Investigator
Advisor: Dr. Norman LaVoie

March, 2000

SECTION B *(this section pertains only to the final 20-25 subjects)*

Based upon your PAP score, you have been asked to volunteer for the remainder of this study. As a reminder, the purpose of this study is to investigate the influence of fiber type distribution on the uptake of supplemental creatine and its effect on repeated bouts of maximal cycle ergometry. The following paragraphs will outline what is expected of each participant for the remainder of the study.

You will be required to record your daily nutritional intake for 3 days on two separate occasions. That is, everything that you consume (food and drink) will be written down in a logbook according the procedures outlined in the information session.

You will also be required to provide, on two separate occasions, urine, blood, and muscle samples. The urine sample requires that you collect all urine for a 24 hour period and return the sample to the laboratory at the specified time. In donating blood, you will be asked to allow a phlebotomist to withdraw 5 ml of venous blood and can expect minimal discomfort with this procedure.

The muscle sample technique was reintroduced by Bergstrom in 1962 and has been used extensively in the area of biochemistry since that time. A medical doctor will, under an aseptic technique, remove a 50-75 mg muscle sample (one-tenth the size of a sugar cube) from a small, 1 cm incision from the vastus lateralis muscle group using a 6 mm biopsy needle under local anesthetic (2-3 ml of xylocaine hydrochloride). There exist the possibility of a hypersensitivity reaction to the local anesthetic, in which slight discomfort could persist for 1 to 2 hours following the procedure. Furthermore, there exist the possibility of slight muscle soreness for few days following the muscle biopsy procedure. In addition, if samples are taken in a highly innervated and vascularized area, there exist the possibility of nerve damage and arterial bleeding. However, the area that will be sampled in this study reduces the probability to near nonexistence.

You also be required to perform, on two separate occasions, 5 repeated bouts of cycle ergometry. More specifically, each bout will be 15 seconds in duration with an active rest period of 45 seconds between bouts. Following this test, you may experience a brief period of muscular fatigue in your legs and possibly nausea. You will also be required to refrain from ingesting food, caffeine, and/or alcohol for three hours prior to the initiation of testing.

As previously stated, you will be required, on two separate occasions, to record your nutritional intake, to provide urine, blood and muscle samples, and to perform repeated bouts of cycle ergometry. In the time separating those occasions, you will receive either 4 doses of 5 grams of creatine monohydrate + 2 grams of dextrose powder for a period of 7 days (treatment) or 4 doses of 7 grams of Dextrose (simple sugar) for a period of 5 days (placebo). The only consistently reported side-effect of short and long-term

creatine supplementation in peer reviewed publications, has been an increase of 0.5 kg - 2.4 kg in body weight. Furthermore there have been unpublished, and therefore ancedotal reports of creatine supplementation resulting in muscle cramping and spasms during high intense exercise.

The potential benefit of this study is to better understand who would benefit most from creatine supplementation. Creatine monohydrate is one of the most widely used nutritional supplements in the past decade and the obtained data should provide answers related to whether or not fiber type composition influences the uptake of creatine, and subsequently effect repeated bouts of cycle ergometry.

As a volunteer, you have the right to refuse any test and to withdraw from the study at any time. All information collected for the purpose of this research will remain confidential through the use of a coding system. No reference to any participants will be made an any report of the results. The data will be stored by Marc Poirier and by Dr. Norman Lavoie within the Faculty of Kinesiology for a period of seven years. The findings of the study will be made available to you at your request upon completion of the project.

If you have any questions concerning the study, I may be reached by phone at home (807)-577-0304 or at work (807)-346-7815. I may also be reached by means of email at *(mpoirier@ice.lakeheadu.ca)*.

Marc Poirier
Principal Investigator
Advisor: Dr. Norman LaVoie

Appendix C

"The Influence of Type II Muscle Fibers on the Uptake of Creatine and its Effect on Repeated Bouts of Cycle Ergometry"

October, 1999

SECTION A:

I _____, agree to perform a single 30 second bout of cycle ergometry as part of the subject selection process for the research study being conducted by Marc Poirier, a graduate student within the faculty of Kinesiology at Lakehead University. I sign this with the knowledge that I may experience a brief period of muscular fatigue in my legs and nausea following the test.

I have read and understand **SECTION A** of the cover letter, which outlines that the purpose of this step in the study is to collect peak anaerobic power (PAP) scores from 150-200 undergraduate, male kinesiology students at Lakehead University. Furthermore, I understand that the final group of 40-50 participants will be asked to volunteer for the study based on their PAP scores and that those individuals not approached will have fulfilled their role in this study.

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date

SECTION B

I _____, agree to participate in the remainder of the study outlined in **SECTION B** of the cover letter. That is, to investigate the influence of muscle fiber type distribution on the uptake of supplemental creatine and its effect on repeated bouts of maximal cycle ergometry..

I _____, furthermore agree to record all my daily nutritional intake for 3 days on two separate occasions. That is, everything that I consume (food and drink) will be written down in a logbook according the procedures outlined in the information session.

I _____, agree to provide, on two separate occasions, urine, blood, and muscle samples as outlined in the accompanying cover letter. I sign this

form with the knowledge that I may experience some minor discomfort when blood and muscle samples are taken.

I _____, also agree to perform, on two separate occasions, 5 repeated bouts of cycle ergometry (15 seconds in duration with an active rest period of 45 seconds between bouts) and that for 3 hours leading up to this test, that I will have refrained from ingesting food, caffeine, and/or alcohol. I sign this form with the knowledge that I may experience a brief period of muscular fatigue in my legs and nausea following the test.

Finally, I _____, agree that I may receive either 4 doses of 5 grams of creatine monohydrate + 2 grams of dextrose powder (sugar) for a period of 5 days (treatment) or 4 doses of 7 grams of dextrose powder (sugar)for a period of 5 days (placebo) as outlined in the accompanying cover letter.

I understand that as a volunteer, I have the right to refuse any test and to withdraw from study at any time. All information collected for the purpose of this research will remain confidential through the use of a coding system. No reference of my participation will be made in an any report of the results. The data will be stored by Marc Poirier and by Dr. Norman Lavoie within the Faculty of Kinesiology for a period of seven years. The findings of the study will be made available to me at my request upon completion of the project.

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date

Appendix D

PERFORMANCE AGREEMENT

I _____(MD), have read the research proposal entitled, "The influence of Type II Muscle Fibers on the Uptake of Creatine and its Effect on Repeated Bouts of Cycle Ergometry" and understand the expectations that are needed of me in this study. I_____, furthermore agree to advise all potential participants of the risks associated with all the procedures outlined in the proposal.

_____ _____

Signature of Medical Doctor Date

_____ _____

Signature of Principle Investigator Date

_____ _____

Signature of Advisor Date

Appendix E

Please take your time in filling out this questionnaire as the information is very important to the results of this study. If you answer NO to any of the questions below, please provided as much information as possible. It is more important that as a researcher, I know exactly what the participants did, even if it was not as instructed.

1. **In consuming the supplementation, did you follow the instructions provided?**

   YES ☐ NO ☐

   If NO, please specify:_____

_____

_____

_____

2. **When collecting the 24 hour urine samples, were you able to collect all the urine as asked?**

   YES ☐ NO ☐

   If NO, please specify:_____

_____

_____

_____

3. **When considering your normal level of physical activity, do you believe it varied significantly (i.e. intensity, frequency, and/or duration) over the time period of this study?**

   YES ☐ NO ☐

   If NO, please specify:_____

_____

_____

_____

4. **Did you accurately fill out the nutritional assessment forms provided to you?**

   YES ☐ NO ☐

   If NO, please specify:_____

_____

_____

_____

5. **Please provide any additional information that you feel is relevant to your participation in this study?**

   YES ☐ NO ☐

   If NO, please specify:_____

_____

_____

_____

Appendix F

Date **Day of the week**

Subject ID No. **Name of Subject**

Place Eaten	Time	Description of Food or Drink	Brand Name	Amount

Table 1.2: Form used to record detailed food intakes.

Appendix G

Table 17. Statistical Data for a 2 x 2 x 2 Factorial ANOVA with body mass as the Dependent Measure.

Source	*SS*	*df*	*MS*	F-value	Significance
WAnT	452.16	1	452.16	1.0	0.33
Treatment	57.01	1	57.01	0.13	0.73
WAnT x Treatment	9.96	1	9.96	0.02	0.88
Error-Between Groups	6723.12	15	448.21	___	___
Time	3.75	1	3.75	7.17	0.02
Time x WAnT	0.03	1	0.03	0.07	0.80
Time x Treatment	0.49	1	0.49	0.94	0.35
Time x WAnT x Treatment	0.24	1	0.24	0.47	0.50
Error-Within Groups	7.84	15	0.52	___	___

Table 18. Statistical Data for a 2 x 2 x 2 Factorial ANOVA with protein intake as the Dependent Measure.

Source	*SS*	*df*	*MS*	F-value	Significance
WAnT	155.34	1	155.34	0.26	0.62
Treatment	107.36	1	107.36	0.18	0.68
WAnT x Treatment	656.47	1	656.47	1.07	0.32
Error-Between Groups	9134.25	15	608.95	___	___
Time	35.29	1	35.29	0.57	0.46
Time x WAnT	32.57	1	32.57	0.52	0.48
Time x Treatment	6.64	1	6.64	0.11	0.75
Time x WAnT x Treatment	4.66	1	4.66	0.08	0.79
Error-Within Groups	932.79	15	62.18	___	___

Table 24 Statistical Data for a 2 x 2 x 5 Factorial ANOVA with PAPr (change score) as the Dependent Measure.

Source	*SS*	*df*	*MS*	F-value	Significance
WAnT	1.36	1	1.36	2.64	0.13
Treatment	0.73	1	0.73	1.42	0.25
WAnT x Treatment	0.16	1	0.16	0.32	0.58
Error-Between Groups	7.73	15	0.52	___	___
Bouts	0.89	2.32	0.38	0.85	0.45
Bouts x WAnT	1.19	2.32	0.51	1.12	0.34
Bouts x Treatment	0.19	2.32	0.08	0.18	0.87
Bouts x WAnT x Treatment	0.37	2.32	0.14	0.31	0.77
Error-Within Groups	15.78	34.73	0.45	___	___

Table 25. Statistical Data for a 2 x 2 x 5 Factorial ANOVA with PAPa (change score) as the dependent measure.

Source	*SS*	*Df*	*MS*	F-value	Significance
WAnT	13785.46	1	13785.46	3.09	0.10
Treatment	10740.31	1	10740.31	2.41	0.14
WAnT x Treatment	1989.37	1	1989.37	0.45	0.51
Error-Between Groups	66835.99	15	4455.73	___	___
Bouts	7076.62	2.18	3247.22	0.97	0.40
Bouts x WAnT	6836.47	2.18	3137.00	0.94	0.41
Bouts x Treatment	1990.70	2.18	913.47	0.27	0.78
Bouts x WAnT x Treatment	3825.18	2.18	1755.24	0.52	0.61
Error-Within Groups	109606.36	32.69	3352.98	___	___

Table 26. Statistical Data for a 2 x 2 x 5 Factorial ANOVA with MAPr (change score) as the dependent measure.

Source	*SS*	*df*	*MS*	F-value	Significance
WAnT	1.54	1	1.54	2.05	0.17
Treatment	0.65	1	0.65	0.87	0.38
WAnT x Treatment	0.30	1	0.30	0.40	0.54
Error-Between Groups	11.25	15	0.75	___	___
Bouts	0.83	4	0.21	1.83	0.13
Bouts x WAnT	0.24	4	0.09	0.53	0.72
Bouts x Treatment	0.18	4	0.06	0.38	0.82
Bouts x WAnT x Treatment	0.43	4	0.17	0.95	0.44
Error-Within Groups	6.79	60	0.18	___	___

Table 27. Statistical Data for a 2 x 2 x 5 Factorial ANOVA with MAPa (change score) as the dependent measure.

Source	*SS*	*df*	*MS*	F-value	Significance
WAnT	17491.29	1	17491.29	2.00	0.18
Treatment	8552.36	1	8552.36	0.98	0.34
WAnT x Treatment	3774.17	1	3774.17	0.43	0.92
Error-Between Groups	130971.27	15	8731.42	___	___
Bouts	4960.61	4	1240.15	1.39	0.25
Bouts x WAnT	3207.13	4	801.78	0.90	0.47
Bouts x Treatment	763.13	4	190.78	0.21	0.93
Bouts x WAnT x Treatment	4303.46	4	1075.86	1.20	0.32
Error-Within Groups	53433.88	60	890.57	___	___

Table 28. Statistical Data for a 2 x 2 x 5 Factorial ANOVA with FI (change score) as the dependent measure.

Source	*SS*	*df*	*MS*	F-value	Significance
WAnT	6.24	1	6.24	0.05	0.82
Treatment	27.19	1	27.19	0.27	0.63
WAnT x Treatment	212.47	1	212.47	1.84	0.19
Error-Between Groups	1728.31	15	115.22	___	___
Bouts	118.77	4	29.69	0.87	0.49
Bouts x WAnT	175.99	4	43.99	1.29	0.28
Bouts x Treatment	6.38	4	1.59	0.05	0.99
Bouts x WAnT x Treatment	66.09	4	16.52	0.49	0.75
Error-Within Groups	2037.74	60	33.96	___	___

Appendix H

It should be noted that when a repeated measures factorial ANOVA is calculated, Mauchly's test of sphericity is performed. If this test is significant, than the probabilities associated with the F value need correction by multiplying the degrees of freedom by the value of Greenhouse-Geisser epsilon (Puri, 1996). Appendix G provides the results of Mauchly's test of sphericity, also known as Mauchly's W, for each of the five dependent variables, with only PAPr and PAPa being significant. Therefore, the appropriate corrections were performed to the degrees of freedom and the probabilities for these dependent measures.

Results of Mauchly's test of Sphericity

	Maulchy's W	Degrees of Freedom	Significance	Greenhouse-Geisser Epsilon
PAPr	.155	9	.003*	.579
PAPa	.141	9	.002*	.545
MAPr	.288	9	.056	.618
MAPa	.354	9	.127	.684
%PD	.399	9	.198	.717

*significant at the 0.05 level