NUTRITIONAL CREATINE SUPPLEMENTATION AND BLOOD GROWTH HORMONE CONCENTRATIONS DURING AEROBIC AND ANAEROBIC EXERCISE

by

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ABSTRACT

NUTRITIONAL CREATINE SUPPLEMENTATION AND THE BLOOD GROWTH HORMONE CONCENTRATIONS DURING AEROBIC AND ANAEROBIC EXERCISE

The purpose of this study was to assess the effects of a 5-day creatine supplementation protocol on the performance and corresponding plasma growth hormone (GH), lactate (LA), and glucose (GL) responses to a supramaximal (125% $\dot{V}O_2$ peak) and submaximal (70% $\dot{V}O_2$ peak) exercise. These results showed that five days of creatine supplementation (4x5g/day) significantly increased time to exhaustion and maximal accumulated oxygen deficit during the supramaximal exercise with no significant differences in GH, LA and GL levels compared to placebo. No significant differences between creatine and placebo supplementation in performance enhancement or GH, LA and GL responses were found in the submaximal exercise protocol, although there was a tendency towards increased GH levels after creatine supplementation. A linkage between the muscle-enhancing effects of creatine supplementation and growth hormone responses could not be established.

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INTRODUCTION

Creatine monohydrate is an ergogenic aid that has received a great deal of attention from both the scientific community and general public over the last decade. It has been reported that creatine supplementation enhances an individual's ability to perform high-intensity, short-duration exercise by increasing the supply of phosphocreatine available in the muscle, by increasing the rate of adenosine triphosphate resynthesis, and through buffering lactic acid accumulation. Other studies suggest that these performance benefits decrease and disappear, as the exercise tends to become more aerobic in nature. The benefits that arise from creatine supplementation can increase performance acutely but more importantly, may lead to increased performance during an extended training program. It is this possibility that makes creatine supplementation so attractive to the general public and high-performance athletes. One of the effects of long-term supplementation in combination with a progressive resistance-training program is an increase in lean body mass (Volek et al., 1999). Recent evidence suggests that this lean body mass increase may be primarily due to increases in muscle hypertrophy. Muscle hypertrophy results from an increase in protein synthesis and genetic transcription at the cellular level. There are many factors that can contribute to a proliferation of protein synthesis and genetic transcription. One of the most powerful influences of these biological processes is human growth hormone (GH). Growth hormone is essential for the normal development of the human body with the largest daily production and release of GH occurring at puberty and lasting into an adult's early 20's. The bulk of daily GH release occurs nocturnally in a pulsatile fashion. Exercise also acts as a strong stimulant to GH release. The pathways that lead to an increase of GH are not absolutely clear.

Recent research suggests that there may be many variables in the control of GH release with exercise intensity/duration, corresponding catecholamine release and increased core temperature garnering the most support. Also receiving some support in the literature is a positive relationship between increased plasma [H+] and increased GH release. With increased strength and mass a desirable benefit of prolonged creatine supplementation (with training), the impetus for these increases comes into question. The literature fails to identify the relationships between creatine supplementation and hormone/metabolite secretion. Knowing that creatine supplementation has previously been shown to increase lean body mass, the underlying hypothesis in this study is that creatine may possibly elevate GH concentrations after supplementation. This study attempts to address some of these gaps in knowledge by evaluating the hormone/metabolite response to both aerobic and anaerobic exercise before and after creatine supplementation.

REVIEW OF LITERATURE

Methylguanidine-acetic acid, commonly known as creatine, is a naturally occurring amino acid in the human body with approximately 95% of it being stored in skeletal muscle. Approximately 66% of the skeletal muscle creatine stores exists as phosphocreatine (PCr). The remainder of the body's creatine supply is scattered throughout the body in the brain, heart and testes. The body acquires approximately 1-2 g of creatine through dietary intake, mostly in the form of red meat. Creatine is also constantly broken down and synthesized endogenously. However, in the absence of any dietary creatine, concentration are maintained at only 70-80% (Culpepper, 1998). Initial synthesis occurs in the kidneys with the combination of glycine and arginine to form guanidine acetate. As this point the compound enters the circulation and travels to the liver where a methyl group from methionine is surrendered to create a creatine molecule.

The creatine molecule, or more specifically the phosphocreatine (PCr) molecule, plays a major role in a muscle's ability to perform and maintain short duration, high intensity exercise. The mechanism by which this is accomplished involves the donation of a high-energy phosphate from PCr to rephosphorylate an adenosine diphosphate molecule (ADP) (Chanutin, 1926). During this type of activity, it is most probable that performance deteriorates from a decrease in PCr stores and subsequently a reduction in readily available ATP (Hultman et al., 1996). This is the rationale for supplementing with creatine in the hope of improving exercise performance. Creatine as an effective ergogenic aid relies on the validity of the hypothesis that supplementation with creatine leads to an increase in muscle creatine and PCr stores which accelerate the resynthesis of ATP from ADP (Greenhaff et al., 1994).

Bioavailability and Effects of Supplementation

An average sized individual (70 kg) carries approximately 120 g of total creatine (TCr – free and phosphorylated) (Harris et al. 1992). The mean concentration of TCr within the muscle is between 120 and 125 mmol/kg dry mass (Balsom et al., 1995; Febbraio et al., 1995). Many studies have demonstrated an increase in TCr and PCr stores due to supplementation. Some early investigators (Harris et al. 1992) determined via muscle biopsy that 5 g of creatine taken 4-6 times/day for 2 days was sufficient enough to significantly elevate TCr content in the quadriceps femoris muscle. Others (Greenhaff et al. 1993) followed a 5g, 4-times/day supplementation phase for 5 days. It was postulated that this dosage increased available PCr stores supporting higher ATP resynthesis rates as demonstrated through lower plasma ammonia concentrations during exercise with creatine supplementation. As well, more recent work (Casey et al. 1996; Vanderberghe et al. 1997) has demonstrated an increase of 15-30% in TCr and an increase of 10-40% in PCr after a 5-day supplementation of 20 g/day of creatine. Supplementation of subjects with 20-30 g/day of creatine (Harris et al. 1992) resulted in increases in TCr of 127 to 149 mmol/kg dry mass and increases in PCr of 67 to 91 mmol/kg dry mass in muscle tissue. These studies, the majority of which utilized muscle biopsy techniques, demonstrated that total muscle creatine could be elevated through a supplementation protocol of 5 g, 4 times/day for 5 days. This has become an accepted standard dosage for creatine supplementation in the majority of the scientific studies reported in the literature.

The literature suggests that there is a minimal increase needed in muscle creatine content to provoke an effect from creatine supplementation. For example, if an individual has a relatively high muscle creatine concentration before supplementation, then performance benefits will not likely become evident after a supplementation period. It has been suggested that an increase of at least 20 mmol/kg dry mass in muscle creatine stores is necessary for a performance benefit from supplementation (Harris et al. 1992; Greenhaff et al. 1994). Individuals that do not increase muscle creatine stores with supplementation and consequently do not increase performance are referred to as "non-responders". As many as 30% of subjects tested may demonstrate a non-response to creatine supplementation (Greenhaff, 1997). Within a normal distribution, some individuals have naturally high concentration of muscle creatine stores. Supplementation for these individuals may not increase TCr sufficiently to elicit a significant increase in performance.

In an attempt to enhance the effects of creatine supplementation, some investigators have studied the effect of creatine loading in combination with other ergogenic aids. Augmentation of creatine supplementation with carbohydrate ingestion (Green et al. 1996) increased muscle creatine stores a further 10% as compared to creatine supplementation alone. This is believed to be due to an insulin-mediated increase of creatine transport into the cell (Steenge et al. 1998) by stimulation of sodium-potassium pump activity. It has been suggested that this mechanism is also active during creatine uptake into the cell. Interestingly, the marriage of creatine and carbohydrate ingestion may have other effects. In exercise-exhausted muscle, glycogen

supercompensation was enhanced by a creatine and carbohydrate mixture as compared to carbohydrate ingestion alone (Robinson et al. 1999)

Performance Effects with Creatine Supplementation

A number of conflicting studies have demonstrated that creatine supplementation does and does not enhance exercise performance. The effectiveness of creatine as an ergogenic aid has been studied across a wide range of exercise mediums and through a large span of exercise intensities and durations. Most of the studies have focused on multiple sets of maximal muscle contractions and repetitive sprints.

A good proportion of the studies that evaluated multiple sets of maximal muscle contractions focused on an assessment of response of the lower limb muscle to creatine supplementation. Subjects supplemented with creatine performing 20 x 30-sec maximal effort isometric muscle contractions with intermittent 16-sec rest periods exhibited a significant increase (11%) in total force production and a 10% increase in maximal force production (Lemon et al. 1995). Similarly, creatine supplementation induced a 10-23% increase in muscle torque production in the execution of three consecutive maximal isometric plantar flexion contractions followed by dozens of maximal effort knee extensions (Vanderberghe et al. 1996). A 5-7% improvement in the first 30 sec of a 45-sec maximal continuous jumping test was found in subjects that had ingested 20 g/day of creatine during the 5 days prior to the post-supplement test (Bosco et al. 1997). The effect of creatine supplementation on 5 sets of 30 maximal knee extension contractions with a 60-sec rest between sets was reflected in significant increases in total peak torque production, especially in the second and third sets of the exercise (Greenhaff et al. 1993).

Similarly, the positive effects of creatine supplementation included an increased maximal strength of 20 to 25% in a test of 30 maximal effort arm flexion contractions (Vanderberghe et al. 1997), and 20 to 35% increases in grip strength performance in test groups supplemented with 30 g/day for 14 days (Kurosawa et al. 1997).

Due to the ease of administration and a high degree of control, the cycle ergometer has been used in many exercise experiments, and many studies have demonstrated a positive ergogenic effect of creatine on cycling performance. The results of creatine supplementation on repeated 6-sec cycle ergometry sprints after administration of dosages of 20-25 g/day of creatine for 5-6 days demonstrated an increased work output for the creatine group with significant differences especially noted during the later sprints and within the 4-6 sec range of each sprint (Balsom et al. 1993, 1995). Creatine supplementation during a 28-day resistance/agility training program, utilizing multiple sets of 6-sec cycle ergometer sprints, resulted in an increase in mean work performance as well as an increase in lifting volume for a number of resistance exercises (Almada et al. 1997; Kreider et al. 1998). Investigations into the outcome of a 20 g/day, 5-day creatine supplementation protocol on multiple 6-sec cycle ergometer sprints separated by 30 sec of rest demonstrated a significant increase in total work performance and peak power (Dawson et al. 1997; Ferreira et al. 1997).

Numerous studies of sprint performance (30 sec cycle ergometer sprints) on the cycle ergometer (Birch et al. 1994; Earnest et al. 1995; Kirskey et al. 1997) have shown

that accepted dosages of creatine during a 4-6 week training program resulted in a 9-23% increase in work performance for the 30-sec Wingate test.

Positive studies addressing the ergogenic benefit of creatine supplementation are not limited to assessments involving cycle ergometry. Subjects participating in a 3 x 100 m freestyle swim protocol interspersed with 60 sec of rest between sets (Grindstaff et al. 1997), supplemented with 21 g/day of creatine during 9 days of training improved their swim performance for all three trials ($p=0.057$) and significantly improved their swim time for the first two sets ($p<0.05$). With respect to shorter swim distances, an investigation of subjects performing 6 x 50 m swims with 180 sec recovery, 10 x 25 swims with 60 sec recovery, and 12 x 100 swims with 150 sec recovery (Leenders et al. 1996) reported significant increases in performance in the 6 x 50 m sets for the group supplemented with creatine. Results from the other sprints displayed tendencies for improvement but were not significant.

The effect of creatine loading on dry-land interval sprints has also been assessed (Harris et al. 1993). The performance of subjects that completed 4 x 300 m maximal sprints with 4 min of recovery and the next day performed 4 x 1000 m sprints with 3 min recovery was significantly increased after 5 days of creatine supplementation (5g/day). The subjects' performance in the last set of the 300 m and 1000 m sprints, their best 300m and 1000 m set, and total 4 x 1000 m time were all significantly improved.

The effect of creatine supplementation on exhaustive treadmill runs ranging from 90 to 600 sec in duration was also analyzed (Earnest et al. 1997). Ingestion of 20 g/day

of creatine for 5 days produced significantly greater work across the 90 to 600 sec exercise duration with the greatest improvements realized with the shorter, higher intensity workloads. Subsequently, the same authors evaluated the 90-sec exhaustive treadmill run. Their experimental design called for two 90 sec exhaustive runs on the treadmill separated by 8 min of recovery. Following a very similar creatine loading phase, total time to exhaustion was significantly extended in the creatine group with the greatest improvement observed in the second set.

Exhaustive exercise protocols utilizing the cycle ergometer have also been used to assess the effect of creatine supplementation on anaerobic power (Jacobs et al. 1997). This study examined the effects of creatine supplementation on an individual's maximum accumulated oxygen deficit (MAOD) (an effective measure of anaerobic capacity) which required each subject to complete an exhaustive cycle ride at 125% of their $\dot{V}O_2$ peak. The subject was then randomly placed in a control or creatine group. The subjects that completed a 20 g/day, 5-day creatine loading phase increased their exercise time significantly by 8% and their MAOD by 9% as compared to the control group. Interestingly, these increases remained elevated when re-tested 7 days after cessation of the supplementation (time to exhaustion 7%; MAOD 7%). A similar protocol (Smith et al. 1998) was used to study the outcome of creatine supplementation on exhaustive cycle ergometry with varying workloads to elicit fatigue within 90 – 600 sec. Anaerobic work capacity (W') was calculated via a non-linear, hyperbolic functional relationship of time to exhaustion vs. work rate (watts). The subject group that underwent creatine supplementation significantly increased the times to exhaustion, thus significantly increasing estimates of anaerobic work capacity derived from the noted relationship. The

improvements in W' were primarily due to increases in time to exhaustion for trials that lasted 90 – 240 sec. The authors attributed these improvements to an enhanced involvement of the anaerobic energy system due to increased PCr stores after supplementation.

Not all studies however, have demonstrated a performance enhancing effect of creatine supplementation. No significant performance differences were apparent after a standard creatine loading protocol on 2 consecutive 700 m sprints (separated by 60 min rest) although there was a tendency towards faster times in the creatine group (Terrillion et al. 1997). Longer duration aerobic exercise has been studied for the potential of creatine supplementation increasing performance. Performance on a 6 km, cross-country run after creatine supplementation resulted in no increase in maximal oxygen uptake and a significant increase ($p < 0.05$) in performance time (Balsom et al. 1993). The authors attribute this to the significant weight gain in the creatine group after supplementation as a possible explanation for the slower performance times. No significant differences in respiratory exchange ratio or blood lactate during incremental exercise were found in subjects who exercised at workloads from 50% to 90% of their $\dot{V}O_2$ max for 6 min each with measurements taken at each level and at 5 min intervals post exercise (Stroud et al. 1994). The assessment of a predominantly aerobic exercise (with anaerobic bursts) in subjects performing 25 km cycle time trials with interspersions of 15 sec maximal sprints at 4 km intervals showed a tendency towards (albeit non-significant) a decrease in exercise times after creatine supplementation (Godley et al. 1997).

Assessment of the effect of creatine supplementation on single-effort sprint performance in elite swimmers did not demonstrate faster swim times in a single-effort

bout (Burke et al. 1996). The study did not measure muscle creatine concentration, which complicates conclusions drawn from the performance data. The authors concede that the rest intervals utilized in the experiment did not reflect rest intervals available during swim meet competitions. Therefore, conclusions on performance during a competition cannot be easily drawn. Another investigation (Mujika et al. 1996) of the effect of creatine supplementation (20 g/day of creatine for 5 days) on swim performance in national and international level swimmers found no increases in performance after creatine supplementation with performance actually deteriorating in some cases. This may be attributed to the fact that the weight gain in the creatine group might have altered the swim mechanics and hydrodynamic drag of the athletes. Also, the fact that the athletes were already performing at an elite level may have created difficulties in assessing improvement in performance.

Creatine supplementation with only 3 g/day for 14 days did not produce any increase in the 1RM bench press, 40-yd sprint or leg extension (Goldberg et al. 1997). This study may have not utilized a long enough supplement phase and/or a great enough loading phase dose to evoke a response. No positive effect of creatine supplementation was found on power output during two consecutive 15 sec maximal power tests (separated by 20 min of rest) (Cooke et al. 1995), or on a 20 sec maximal sprint on an air-braked cycle ergometer after 5 days of 30g /day of creatine supplementation (Snow et al. 1998).

Other investigators (Odland et al. 1994; Ruden et al. 1996) did not observe any significant performance increases in a 30 sec maximal effort cycle ergometer test. These studies utilized a crossover design but only allowed for a 14-day washout period to allow

creatine concentration to normalize. It is accepted (Febbraio et al. 1995) that it most likely takes closer to 28 days or even 35 days (Lemon et al. 1995) to normalize creatine concentration upon cessation of supplementation. One experiment on the effect of creatine supplementation (followed by a 28-day washout period to normalize intramuscular creatine concentration) on intermittent, supramaximal exercise performance (Febbraio et al. 1995) involved four 1-minute exercise bouts at 125% $\dot{V}O_2$max with 1-minute rest intervals concluding with an exercise bout to exhaustion. The elevated muscle TCr concentration after supplementation did not elicit an increase in performance when compared to the control condition. The investigators used an invasive measure (muscle biopsy) to determine TCr concentration. The only drawback with this study was that only 6 subjects were tested which might have impacted the results.

Through analysis of both aerobic and anaerobic exercises as well as a variety of exercise mediums, the consensus in the literature indicates that creatine supplementation produces an enhancement of high-intensity, short-duration intermittent work – best characterized as anaerobic in nature. Studies that involve more aerobic exercise with creatine supplementation did not elicit a performance effect from creatine supplementation.

Mechanisms of Action

Davies (1965) first described the role of the phosphogen system in muscle contractions stating that the importance of the phosphogen system lies in its ability to maintain a high intracellular adenosine triphosphate:adenosine diphosphate (ATP:ADP) ratio. With PCr readily available in the cell, ADP and H^+ production is buffered by PCr

donating its high-energy phosphate bond (Bergman et al. 1985). The development of fatigue during short-term, maximal exercise is due to the inability of depleting PCr stores to maintain a high ATP:ADP ratio. Many theories have been proposed as to the mechanism of action that enables heightened creatine concentration to influence performance. With an increase in PCr and TCr from creatine supplementation (Harris et al. 1992; Greenhaff et al. 1993), it is thought that initial elevated PCr stores will allow more work to be accomplished by maintaining a high rate of ATP resynthesis (Casey et al. 1996). For short duration exercise, increased PCr concentration contribute to lower lactate (therefore lower $[H^+]$) accumulations in exercising muscle (Balsom et al. 1995). Another benefit that has been observed is that the abundance of free creatine lends itself to an increased rate of PCr resynthesis (Greenhaff et al. 1994). In general, higher initial concentration of PCr allow more work to be accomplished before the detrimental effects of depleted concentration hinder exercise and an increased rate of synthesis of PCr between subsequent bouts of intermittent exercise allows for faster recovery and more overall work to be performed.

Until 1999, much of the literature supported the theories previously mentioned. In a study that monitored intramuscular PCr concentration during exercise with nuclear magnetic resonance (NMR) imaging (Vandenberghe et al. 1999), an increased rate of synthesis of PCr was not observed in spite of increased TCr concentration due to supplementation. This study is interesting in that it somewhat clouds the issue on what was thought to be a well understood mechanism of action with creatine supplementation. Recent investigators (Van Leemputte et al. 1999) have approached this problem from a

slightly different viewpoint by studying the contractile properties of exercising muscle before and after creatine supplementation. They discovered that these contractile properties were affected by creatine supplementation and consequently, that the relaxation time between contractions was increased by 20%. They suggest that this increased relaxation time during exercise contributes to the delay in muscle fatigue during exercise. More specifically, the authors state that increased PCr stores after supplementation inhibit the accumulation of ADP concentrations and this in turn maintains the efficiency of Ca^{2+}-ATPase to regulate muscle relaxation time.

Side Effects of Creatine Supplementation

By following a standard diet, 1-2 g of creatine are ingested daily (via red meat, fish or chicken). The body supplements any further requirements by endogenously synthesizing creatine. Creatine intake and synthesis are necessary because creatine is constantly excreted by the kidneys after it is non-enzymatically dehydrated to a cyclic creatinine molecule at a rate proportionate to total body muscle mass (Walker, 1979). This constant rate of urinary excretion of creatinine (approximately 25 mg/kg/day) serves as an efficient method of estimating renal function. By increasing creatine (amino acid based molecule) through supplementation, concerns are raised about the known effect of high-protein intake on kidney function and its relation to progressive dysfunction in previously diseased kidneys (Brenner et al. 1982). A standard creatine loading protocol (20 g/day for 5 days) induced a four-fold increase in arterial creatine concentration and an increase from <150 mg/day to almost 13 g/day of urinary creatine (Poortmans et al. 1997). However, over this 5-day period there was only a negligible increase in urinary

creatinine concentration. Other studies evaluated the long-term effect of creatine supplementation. Creatine supplementation protocols consisting of at least 10 g/day for 56 days (Almada et al. 1996; Earnest et al. 1996) and for 365 days (Sipila et al. 1981) demonstrated only slight increases in serum creatinine concentration. It is hypothesized that the increases in urine and serum creatinine with creatine supplementation are probably due to an increased release and recycling of intramuscular creatine. This increased response may be due to enhanced myofibrillar protein turnover and not due to renal pathology (Balsom et al. 1994; Earnest et al. 1996). The studies referred to previously involved subjects with normal renal function. Professional opinion implies that metabolizing abnormally higher amounts of creatine may further hinder individuals with pre-existing diminished kidney function.

Another side effect of creatine supplementation that has been well documented in the literature is that of weight gain. Studies using a short-term creatine supplementation period (20 g/day for 5 days) have demonstrated increases in body mass ranging from 0.9 to 1.6 kg (Balsom et al. 1993; Greenhaff et al. 1994; Vandenberghe et al. 1996). This significant weight gain over such a short period of time has been theorized to be intracellular water retention associated with creatine storage (Balsom et al. 1994; Ziegenfuss et al. 1997). A number of long-term studies have demonstrated equal or greater increases in body mass after supplementation. With longer duration studies, the weight gain has been attributed to increases in lean body mass as well as water retention. A 4.8 kg increase in fat-free mass has been observed with a 0.3 g/kg/day creatine regimen during a 42-day pre-season conditioning program (Kirksey et al. 1997). A 60% increase in fat-free mass was observed in 19 females undergoing creatine supplementation with 20

g/day for 4 days and 5 g/day for an additional 66 days (Vanderberghe et al. 1997). These subjects trained throughout the supplementation period with the creatine group demonstrating a 25% greater increase in maximal strength. Interestingly, after a 28-day washout period, PCr concentration declined while gains in fat-free mass were preserved.

These increases in fat-free mass are well documented but it has only been recently that clarification has come in terms of changes in muscle morphology that may lead to mass gains with creatine supplementation. A recent study (Volek et al. 1999) measured the effect of creatine supplementation on muscle strength, fat-free mass, and muscle fibre cross-sectional area over a 12-week training program. Subjects receiving creatine demonstrated greater increases in maximum strength and fat-free mass. Also, creatine subjects experienced a greater increase in Type I (35% vs. 11%), Type IIA (36% vs. 15%) and Type IIAB (35% vs. 6%) muscle fibre cross-sectional area as compared to a placebo group. This increase translated into a significantly higher training volume for the creatine group, especially during the last 4 weeks of the 8-week training session. The authors of this study attributed the greater muscle hypertrophy to higher quality individual training sessions from the enhanced creatine concentration that led to accelerated physiological adaptations to the training.

This finding suggests that during this 12-week training and supplementation period, there was an increased rate of protein synthesis in the muscle of the subjects that received creatine supplements. There are many factors that affect protein synthesis. If the contention of Volek and coworkers (1999) is that better quality training sessions are achieved while supplementing with creatine, then how exactly are these training sessions stimulating more protein synthesis? One of the major hormones that is greatly affected

by exercise and is major factor in protein synthesis and genetic transcription is growth hormone.

Metabolite Changes with Creatine Supplementation

Throughout the documented research, metabolite changes have not been assessed extensively with respect to creatine supplementation. The metabolites assessed in the present study were blood lactate, plasma glucose, and plasma growth hormone concentration. Of these three, the effect of creatine supplementation on lactate accumulation during exercise is the most documented and best understood.

Lactate production during exercise is assessed in one of two ways: through blood sampling for plasma lactate concentration and through muscle biopsy for muscle lactate concentrations. With creatine supplementation, studies have demonstrated an increase, no change in, or a decrease in lactate accumulation in both blood and muscle during exercise. A review of the literature indicates that the variation in the results may be dependent on the type and intensity of exercise utilized in the respective investigations. For example, blood lactate accumulation did not change significantly despite increases in overall work output during five 30-sec maximal isokinetic contractions before and after creatine supplementation (Greenhaff et al. 1993). These authors suggested that the increase in work performance was supported by energy sources other than glycogenolysis. Others (Stroud et al. 1994) studied the effect of creatine supplementation during steady state, incremental exercise on blood lactate accumulation. Lactate concentration was measured while subjects performed exercise at 50% of $\dot{V}O_2$ max and increasing to 90% $\dot{V}O_2$ max. No significant differences were evident. However, there

was a tendency for lower lactate accumulations at the high-intensity range of exercise (90% $\dot{V}O_2$ max) in the creatine group. The effect of supplementation with creatine on all-out 25m, 50m and 100m swims (Burke et al. 1996) did not result in any significant differences in blood lactate although there were tendencies for lower accumulation after supplementation with the 100m swim which demonstrated the closest significant interaction effect ($p = .06$). Another evaluation of swimmers (Mujika et al. 1996) incorporated an almost identical experimental protocol as used by Burke's group and reported very similar findings. Their findings again did not convey any significant change in blood lactate accumulation but a tendency for lower accumulations was again present, especially with the 100m sprint. Measurement of muscle lactate responses to an intense, electrically evoked contraction before and after creatine supplementation did not demonstrate any significant difference between the creatine and placebo conditions (Greenhaff et al. 1994). Also, muscle lactate accumulation before and after four intermittent bouts of supramaximal exercise did not vary significantly between the control and creatine sessions, although there was a tendency towards lower muscle lactate accumulations after exercise during the creatine supplementation phase of the experiment (Febbraio et al. 1995).

Other studies that measured both plasma and muscle lactate accumulation after exercise, demonstrated a significant change with creatine supplementation. Studies of the effect of creatine supplementation on an exhaustive treadmill run lasting 3-6 min as well as a 6-km cross country run reported that the creatine group demonstrated a significant increase in blood lactate during the treadmill run to exhaustion (Balsom et al. 1993). Also reported was a significant increase in time to completion for the 6 km run, which

was related by the investigators to an increase in body mass after creatine supplementation. In 1995, the same authors implemented an exercise protocol that involved a series of 6 sec maximal exercise bouts separated by 30 sec of rest. The subjects maintained a target speed on the cycle ergometer to ensure the same amount of work was performed for each trial. Subjects supplemented with creatine demonstrated a significantly lower muscle lactate accumulation as compared to exercise without creatine supplementation. In another study which assessed all-out performance on a treadmill at an intensity to elicit volitional fatigue at approximately 60 sec (Bosco et al. 1997), a significant increase in blood lactate accumulation after exercise was found in the creatine group which corresponded to a significant increase in time to exhaustion for that same group.

Although the evidence is not unequivocal, a trend in the literature tends to associate creatine supplementation with an effect on lactate accumulation. For repeated bouts of exercise at the same relative intensity, creatine supplementation seems to suppress the accumulation of lactate. Depending on how much more work is accomplished with creatine supplementation, lactate accumulation is the same or increased with maximal exercise to exhaustion. The tendency is for a statistically unchanged lactate concentration despite more work being accomplished or for an increased lactate concentration corresponding to very significant increase in exercise performance.

Unlike the lactate response to creatine supplementation, almost no mention of the glucose and growth hormone responses to exercise are made in the literature as it pertains to creatine supplementation. The only mention of a glucose or growth hormone response

is one study (Vorobiev et al. 1996) in which either a placebo or PCr was administered to subjects 24 h and 30 min before engaging in one of two exercise protocols. The first protocol consisted of an incremental exercise to exhaustion while the second consisted of 35 min of submaximal exercise at 70% $\dot{V}O_2$ max. The glucose and growth hormone responses for the creatine group were not significantly different from the placebo group for both maximal and submaximal exercise. For both types of exercise, there was a trend (albeit non-significant) towards a blunted growth hormone response to exercise with creatine supplementation. Some drawbacks to this study that could lead to possible further investigation include the fact that the investigators did not use a standard creatine loading protocol in their study and that the number of subjects incorporated was only eight. In considering the increases in lean body mass associated with creatine supplementation and the role of growth hormone in the body, further investigation of the interaction of creatine supplementation and the growth hormone response during exercise is warranted.

Growth Hormone

Structure and Secretion

Human growth hormone (hGH) or somatotropin is a 191-amino acid polypeptide that is one of six major anterior pituitary hormones. Growth hormone is a single chain molecule with two intra-molecular disulphide bonds that bind positions 53 and 65 as well as positions 182 and 189 on the amino acid chain. Somatotropic cells in the Golgi area synthesize human growth hormone. After the molecule is formed it is stored in small

granules and when conditions permit, it is released through exocytosis and eventually makes it way into the pituitary portal blood circulation (Draznin et al. 1988).

The stimuli that initiate GH release originate in the hypothalamus. Two hypothalamic hormones, growth hormone releasing hormone (GHRH) and growth hormone inhibiting hormone (GHIH or somatostatin) exert their effects on the anterior pituitary gland. GHRH effects both production and release of GH, while somatostatin only inhibits the release of GH. Under normal conditions, the bulk of GH release occurs during the first non-REM stage of sleep, which is usually during the early morning hours (Kern et al. 1995). This spiked release of GH coincides with a reduction in somatostatin concentration (Hartman et al. 1993). Somatostatin accomplishes this inhibition of GH release by blocking the cAMP messenger system that triggers the release of GH (Postel-Vinay et al. 1996).

The bulk of GH release into the body occurs in spikes. Growth hormone in its free-floating form is broken down and recycled with a half-life of approximately 16 min (Lassarre et al. 1974). As GH is released into the blood plasma, approximately 50% of the plasma GH molecules are bound to growth hormone binding protein (GHBP). Two of these binding proteins have been identified in humans: both a high-affinity and a low affinity GHBP (Baumann and Mercado 1993). It is understood that the GHBP is actually the extracellular region of the growth hormone receptor. In humans, the extracellular portion of the GH receptor undergoes a proteolytic cleavage to become GHBP (Leung et al. 1987). These binding proteins act to prolong the half-life of GH and govern the GH distribution throughout the body. This is an important aspect of the release of GH into the body. GHBP is effective in that it binds to GH and extends the half-life of the

hormone allowing a release of GH to exert its biological effects for a longer period of time.

Growth Hormone Secretion Patterns

The pattern of normal GH release in humans has been extensively studied. Daily growth hormone secretions decrease immediately after birth and are released predominantly via nocturnal pulses throughout early childhood (Albertsson-Wilkand 1988). The growth spurt children experience at puberty results from an increase in both pulse frequency and pulse amplitude with the majority of GH mediated growth due to increases in amplitude instead of an increase in the frequency of pulses (Martha et al. 1989). After the age of 20, GH production decreases to 1 mg/day and to 0.5 mg by the age of the forty. The majority of this release is still nocturnal during a state of deep sleep (Finklestein et al. 1972). As alluded to earlier, a decrease in somatostatin is responsible for the pulsatile release in GH. Variance in somatostatin concentration affects both the timing and duration of GH release (Kracier et al. 1988), while the circulating amount of GHRH is responsible for the amplitude of GH secretion (Brook et al. 1988).

Exercise and Growth Hormone Secretion

Plasma GH concentration increases as a response to stressful situations. Compared to other hormones, GH has a relatively long half-life of approximately 16 min (Lassarre et al. 1974). If exercise is rigorous enough to stimulate an increase in serum GH concentration, a further decrease in the already low elimination rate would not explain the accumulation. Therefore, there must be an increase in secretion to account for higher plasma GH concentration (Galbo 1983).

Early work (Lassarre et al. 1974) reported on the kinetics of human GH during submaximal exercise. Measurements of GH before, during and after 1 h of submaximal exercise demonstrated that there was a delayed GH response of approximately 15 min into exercise followed by a gradual increase in plasma GH until cessation of exercise. Growth hormone concentration decreased exponentially (half-life of 16 min) during the recovery period. Investigation of the effect of manipulating blood [H+] on GH response during exercise (Sutton et al. 1976) revealed a proportionate response of GH to exercise intensity. This response was independent of variations in blood [H+] and lactate concentrations. A study of the effect of ambient temperature on GH response during exercise showed that a walking protocol of 3.5 mph at an 8.6% grade elicited a significant GH response at 40°C (Frewin et al. 1976) which was almost nullified when the same workload was performed at 10°C. Another study reported the lack of a relationship between lactate values (anaerobiosis) and plasma GH responses to exercise (Karagiorgos et al. 1979). The results of this study demonstrated that by controlling for total amount of work accomplished, intermittent exercise at twice the intensity of continuous exercise demonstrated a non-significant tendency towards eliciting higher GH plasma values. No correlation was found between lactate or rectal temperature and GH.

Galbo (1981) suggested that the GH response to exercise is delayed and that this delay is inversely proportionate to exercise intensity. His synopsis of earlier work demonstrates evidence that the GH response may be suppressed during maximal exercise as compared to sub-maximal exercise. As well, the author cited evidence of the GH response to exercise waning after prolonged intermittent or continuous exercise.

These comments have been substantiated in a study of hormonal responses to different types of exercises (Kindermann et al. 1982). Supramaximal exercise (156% $\dot{V}O_2$ max) elicited a very small increase in GH whereas a somewhat greater response was recorded with progressive exercise to exhaustion. The greatest GH response was noted with prolonged aerobic exercise near the anaerobic threshold. Values of approximately 32 ng/mL were recorded at the end of the 50 min exercise period.

It is evident from the literature that the magnitude of the release of GH during exercise is affected by the physical fitness of the subjects, the intensity and duration of the exercise, the type of exercise (Vanhelder et al. 1984a), and the absolute concentration of power output (Sutton et al. 1976). Whereas some workers have reported higher GH responses to aerobic exercise (Kinderman et al. 1982), others have found no differences between intermittent and continuous exercise (Karagiorgos et al. 1979). However, in many studies that compared one type of exercise against another, the exercises were not of equal duration or equal workload. To examine this problem, Vanhelder and coworkers initiated a series of studies that standardized the duration and work output of the exercises compared (Vanhelder et al. 1984a, 1984b, 1985, 1986).

An intermittent cycling anaerobic exercise induced a significantly higher GH response than a continuous aerobic exercise of equal duration and total work expenditure (VanHelder et al. 1984a). Evaluation of two different types of aerobic exercise with equivalent oxygen demands, lactate production and duration did not elicit any significant differences in GH response (VanHelder et al.1986).

In a retrospective examination of previously published data (Vanhelder et al. 1987), VanHelder and coworkers demonstrated the existence of a significant correlation between exercise-induced changes in plasma GH concentration and oxygen demand and availability, expressed as oxygen demand/availability ratio. This relationship held over a wide variety of exercises, aerobic and anaerobic, continuous and intermittent, exercise type, and in both fit and unfit subjects under normoxic and hypoxic conditions. They concluded that differences in oxygen demand and supply were important regulators of GH secretion during exercise.

A study of submaximal and exhaustive exercise reported similar GH responses to both types of exercise (Barreca et al. 1988) with the authors suggesting a relationship between catecholamines and GH responses as well as with lactate.

A study of different resistance protocols showed that a repeated 10 RM protocol promoted significant increases in serum GH compared to the lack of an significant increase by a repeated 1 RM protocol (Hakkinen and Pakarinen 1993). The authors also reported a significant correlation with lactate concentration and GH during and after exercise.

Subjects performing an exhaustive interval treadmill protocol consisting of approximately 15 min of exercise demonstrated increased plasma GH concentration that were apparently intensity dependent (Gray et al. 1993). The investigators suggested that the GH response was related to the intensity of exercise and that there may be a threshold of intensity necessary to promote a significant GH response (Bunt et al. 1986). Others (Gray et al. 1993) have proposed that low blood glucose and increased lactate/reduced pH

may have an influence on the GH response to exercise. The effect of pH manipulation on the GH response to high-intensity exercise was examined in a double-blind, cross-over protocol in which subjects ingested either a placebo or sodium bicarbonate before exercise (Gordon et al. 1994). Measurements of GH after exercise and into recovery indicated that the plasma GH concentration were negatively correlated to pH concentration suggesting that increased [H+] may be at least in part a factor in the control of the GH response to exercise.

It has recently been reported that GH secretion mirrors lactate and catecholamine release and that significant GH increases in serum were only observed once the lactate threshold had been reached (Chwalbinska-Moneta et al. 1996). This study adds to the circumstantial evidence that GH is dependent on an oxygen demand/availability ratio.

A study of inter-subject variability in GH release during different types of work, submaximal exercise intensities, and ambient exercise temperature (Raynaud et al. 1983) reported that GH concentration increased with exercise intensity at 24 °C. This pattern remained consistent with exercise at 33 °C. However, the lowest exercise intensity at the higher ambient temperature produced a greater GH response than that of the highest exercise intensity at the lower temperature. A study of the effect of heating and central cooling on GH response in normal resting males (Weeke and Gundersen 1983) has shown that changes in body temperature have an effect on GH release. Immersion in 31.7°C water while ingesting ice virtually suppressed the GH response while immersion in 39.3°C water stimulated a significant increase in plasma GH. Although not directly

involving exercise, these types of studies have aided in the elucidation of the stimuli involved in the control of GH release.

A comparison of the GH responses induced by passive heating to that of exercise-induced increases in body temperature (Christensen et al. 1984) reported that exercise at an ambient temperature of 22°C provoked a 1°C increase in core temperature with a subsequent increase in GH plasma concentration. Passive heating by exposure to water-filled warming blankets (67°C) for a period sufficient enough to increase body temperature by 1°C also elevated plasma GH. Plasma GH concentration were higher in the passive heating condition even though the rate of temperature increase was twice as large in the exercise condition than in the heating condition. The investigators found that if the exercise was performed in an ambient temperature of 4°C, the GH response was completely inhibited.

The role of increases in body temperature during exercise on the growth hormone response was further studied by employing a thermal clamp technique (Cross et al. 1996; Radomski et al. 1998). This involved exercising in warm or cold water, which is a more effective manner to control body temperature than changing the ambient air temperature. An endurance exercise bout was performed during chest-high immersion in either cold or warm water. Exercise in the warm water resulted in an increase in body temperature with a concurrent increase in plasma GH. Conducting the same exercise in cold water resulted in a clamping of the increase in body temperature and no increase in GH. This work solidified the argument that there is a temperature threshold that must be surpassed to elicit a GH response.

The GH response in sprint-trained vs. endurance-trained athletes was examined during a 30 s treadmill sprint (Nevill et al. 1996). The serum GH response was higher in sprint-trained athletes as compared to endurance-trained athletes. Also, GH concentrations were higher in sprint-trained subjects after 1 h of recovery, which corresponded with the higher lactate concentrations and lower pH concentration. The authors suggest that the increased GH response in sprint-trained athletes was related to higher power outputs during the exercise (due to greater muscle mass) which supports the argument that GH response may be associated with exercise intensity. The authors further submitted that athletes who perform this type of training on a continuous basis might be exposed to chronically higher GH concentration, which could contribute to a greater rate of protein synthesis and protein sparing.

Other work (Weltman et al. 1997) evaluated the GH response to an acute bout of constant-load exercise after a 6-week training period. Even after 3 weeks, the GH response was significantly suppressed at an identical workload after training. The response was almost completely nullified after 6 weeks of training. The training altered the relative intensity of the exercise and consequently the percentage of $\dot{V}O_2$ max that the individuals were working at. The authors concluded that there might be an intensity threshold that must be reached to elicit a GH response and that threshold may correspond to the 50% $\dot{V}O_2$ max proposed by Viru (1985).

The metabolic role of GH in the body is understood to benefit long duration activity with increased lipolysis and to increase protein synthesis and genetic transcription. An increase in GH both during and after exercise is metabolically

beneficial to the initial performance and subsequent performances. The literature to date has evaluated the GH response to exercise with a large body of the work suggesting that GH response may be related to the intensity/duration of the exercise, increases in core temperature, and catecholamine concentration increases during exercise. This knowledge combined with the understanding to date of the influences and benefits of creatine supplementation on exercise poses some interesting questions when considered together. The deficiencies in the literature lie in the lack of information on any possible effects of creatine supplementation on growth hormone secretion as well as the relationship to metabolic changes during different types of exercise after creatine supplementation. The suggestion in the literature that GH response during exercise may be related to intensity and duration is of particular interest in that creatine has been reported to increase the intensity and duration of anaerobic exercise. This combined with the potential of creatine to buffer lactate accumulation, creates an interesting situation when GH response is assessed in combination with creatine supplementation.

In our present study, we investigated the effect of a standard 5-day creatine supplementation protocol on the plasma growth hormone, glucose and lactate responses to supramaximal (125% $\dot{V}O_2$ max) and submaximal (70% $\dot{V}O_2$ max) exercise. The purpose of the study was to assess the possible effects of creatine supplementation on the ability to perform at two exercise intensities and the subsequent effect on the GH, lactate and glucose responses to exercise. We also attempted to verify the positive effect of creatine supplementation on maximum accumulated oxygen deficit as an indirect measure of creatine uptake. This is beneficial in that the ergogenic benefit of creatine on

MAOD has been established (Jacobs et al., 1997) and a creatine loading effect can be determined without the invasive use of a muscle biopsy.

Study Objectives

Working objectives for this study are:

1. To affirm that a 5-day creatine supplementation period increases the maximum accumulated oxygen deficit during short-term, supramaximal exercise with benefits equaling 6 weeks of training.
2. To assess that if creatine supplementation increases the duration of anaerobic work performed, a duration/intensity increase in GH secretion may occur after creatine supplementation.
3. To determine if creatine supplementation affects the metabolic response to aerobic and anaerobic exercise with respect to lactate, glucose and GH.

MATERIALS AND METHODS

Subjects

The Human Ethics Committees of both the University of Toronto and DCIEM approved the protocol for this experiment. Twenty healthy, physically active male subjects between the ages of 18 and 30 y were selected with the aid of a poster from DCIEM, Garrison Support Unit Toronto and the University Community. The design of the experiment demanded five continuous weeks of participation, which made the inclusion of females in the study logistically impossible due to the fact that GH secretions fluctuate by as much as 50% in young women during the stages of the menstrual cycle. The subjects' first visit to the laboratory included a thorough medical screening to ensure that a certain level of general health existed in the subjects before participating in the study. Parameters for screening the subjects mainly centred on cardiac limitations to maximal exercise with due attention also given to pulmonary, musculoskeletal and any other possible limiting factors. After medical screening, all subjects were informed as to the details of the experiment and discomfort and risks associated with the experiment. Subjects were not trained cyclists and they were asked not to engage in physical exercise for 48 h before each testing session. Subjects were not presently taking any creatine supplements or had not taken creatine supplements in the last 30 days.

The subjects' age was 22.7 (± 3.0) yr (mean ± SD) (Table 1). The mean weight of the subjects was 73.5 ± 7.9 kg (mean ± SD) and the mean height was 177.5 ± 5.9 cm (mean ± SD). Percentage body fatness was calculated via a five skinfold body composition assessment method that is utilized by the Canadian Forces (as described by Forsyth et al., 1984). The mean percentage body fat of the subjects was determined to be

13.1 ± 3.5 %(mean ± SD). Lean body mass was calculated by expressing the subjects' percentage body fat as an absolute mass value (% BF x total mass). This value was than subtracted from the total mass value for an estimate of lean body mass. Lean body mass for the subjects was 63.8 ± 6.7 kg.

Table 1: Subject Characteristics

Group	Age (y)	Height (cm)	Mass (kg)	VO2 max ($L \cdot min^{-1}$)	VO2 max ($mL \cdot kg^{-1} \cdot min^{-1}$)	% Body Fat	Lean Body Mass (kg)
C1	21	184.0	73.0	4.0	54.9	7.2	67.7
C2	21	180.0	95.0	4.3	45.6	16.1	79.7
C3	30	162.5	64.6	3.6	55.1	18.0	53.0
C4	22	179.0	72.0	2.9	40.6	16.4	60.2
C5	20	181.0	70.1	4.0	57.1	10.1	63.0
C6	22	174.0	72.0	4.4	61.7	8.4	66.0
C7	25	166.0	62.9	3.3	52.1	14.1	54.0
C8	20	179.0	78.0	4.4	56.2	9.9	70.3
C9	23	180.0	77.5	3.6	46.3	15.7	65.3
C10	29	183.0	80.4	4.5	56.2	11.6	71.1
P1	21	171.5	65.3	3.4	52.7	15.5	55.2
P2	20	177.0	65.0	3.7	56.9	8.3	59.6
P3	25	174.0	72.5	4.1	57.1	9.8	65.4
P4	22	176.0	72.0	3.8	52.5	18.1	59.0
P5	20	185.0	83.2	3.3	39.7	16.6	69.4
P6	21	173.0	68.5	3.0	44.2	11.8	60.4
P7	21	181.0	82.5	3.9	47.2	13.1	71.7
P8	21	181.5	67.0	3.2	47.9	10.0	60.3
P9	27	184.0	78.5	4.0	51.2	16.1	65.9
P10	23	179.0	69.7	4.4	62.7	14.3	59.7
Mean ± SD	22.7 ± 30	177.5± 5.9	73.5 ± 7.9	3.8 ± 0.5	52 ± 6.2	13.1± 3.5	63.8 ± 6.7

Abbreviations: C – creatine-supplemented; P - placebo-supplemented.

Aerobic Power Determination

Subjects' maximal aerobic power ($\dot{V}O_{2\,max}$) was assessed via a progressive resistance protocol on the cycle ergometer. During the initial stages of $\dot{V}O_2$ max assessment the subjects performed submaximal, steady state exercise for 4 min at four different workloads. Expired air was collected and analyzed (Metek gas analysis system)

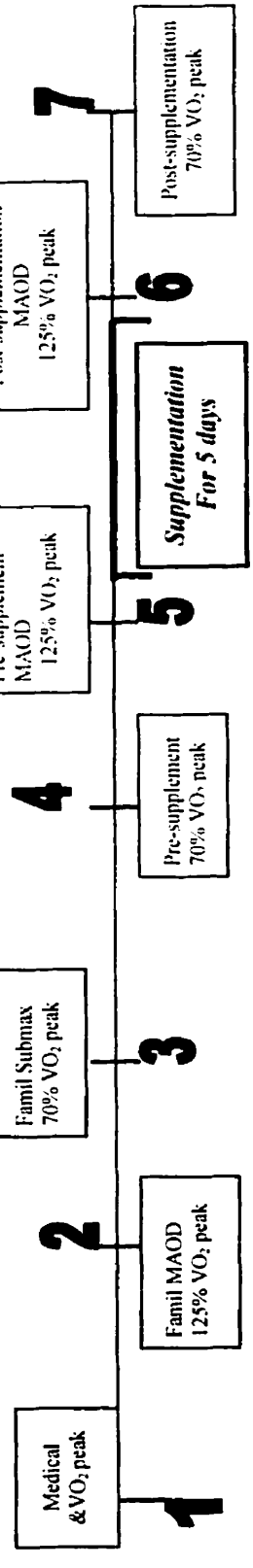
for oxygen and carbon dioxide fractions. Oxygen consumption was recorded at 90W, 120W, 150W, and 180W and a linear regression equation was calculated for each individual to determine a workload value representing 70% and 125% of each individual's $\dot{V}O_2$ max (Table 2). At the conclusion of the four submaximal workloads, the intensity was increased by 30 W/min and $\dot{V}O_2$ max was assessed by the highest average oxygen consumption attained in the last 2 min of the test. Verbal encouragement was given to the subjects during the later stages of the test to elicit a maximal effort. Maximal heart rates were measured by using a transmitter/telemetry heart rate monitor unit (Polar Electro PE3000). The mean $\dot{V}O_2$ max relative to body weight for the subjects was 52 ± 6.2 mL/kg/min (mean ± SD) while the mean absolute oxygen consumption was 3.81 ± 0.47 L/min (mean ± SD) (Table 1).

Experimental Design – Overview

Upon completion of the initial medical screening and $\dot{V}O_2$ max testing the subjects were required come to the laboratory for six additional visits (see Figure 1 for timeline). The objective of the experimental design was to examine the response of growth hormone (as well as other blood markers) during aerobic exercise (70% $\dot{V}O_2$ max) after creatine supplementation. An anaerobic exercise test (125% $\dot{V}O_2$ max) was inserted into the middle of the experimental design to act as a non-invasive measure of the effectiveness of creatine loading in the subjects. The first visit consisted of a medical exam and measurement of the $\dot{V}O_2$ max, visits 2 and 3 of familiarization trials of exercise at 125% [maximum accumulated oxygen test (MAOD)] and 70% of the individuals' $\dot{V}O_2$ max, respectively. The individual workloads for each subject were arrived at via a calculation utilizing a linear regression equation (Table 2). The 3rd and 4th visits were

pre-supplementation, submaximal and supramaximal exercise sessions, respectively. At this point, the subjects were randomly assigned in double-blind fashion to either a placebo or creatine group. The subjects then received supplementation of either a polycose/sucrose placebo or a polycose/creatine/sucrose mixture for five days. Upon completion of the supplementation, the subjects returned to the laboratory and repeated the 125% $\dot{V}O_2$ max (MAOD) test. Within three days following this test, the subjects completed the last post-supplementation, 45-min submaximal test at 70% $\dot{V}O_2$ max.

Figure 1. Experimental Chronology



Time Between Visits: Session 1 and Session 2 – 48 hrs
Session 2 and Session 3 – 48 hrs
Session 3 and Session 4 – 48 hrs
Session 4 and Session 5 – 48 hrs
Session 5 and Session 6 – 5 days
Session 6 and Session 7 – 48 hrs

Standardized Meals

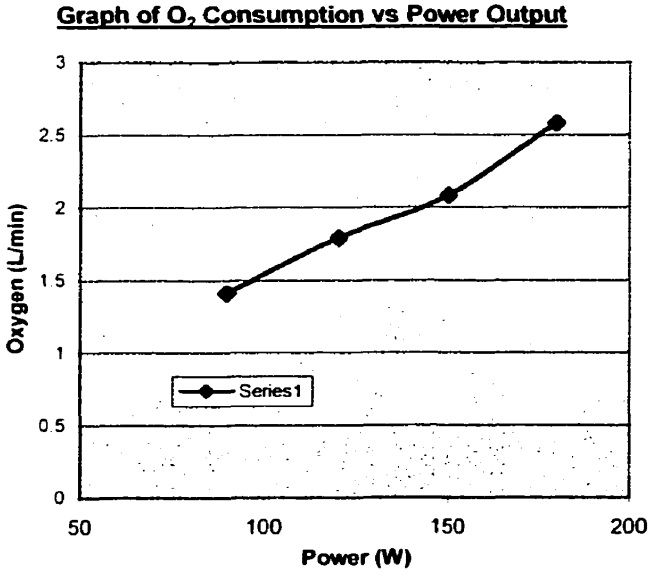
Before the two pre-supplementation exercise sessions and two post-supplementation exercise sessions, the subjects all ingested a standardized meal 90 min before the exercise session. All subjects received a bagel, 250 mL of 2% milk and a 250 mL juice 1 h before a catheter was inserted in the antecubital region of the left arm. If the subjects exercised in the morning, they came to the laboratory in a fasted state not having eaten since the prior evening. If the subjects exercised in the afternoon, they were encouraged to not eat after 08:00 h that morning and to only ingest a light breakfast consisting of toast, juice and milk. Due to time constraints on the part of the participants, not all of the exercise sessions for the study could be completed in the morning.

Table 2: Sample Workload Calculation

Subject exercises at 4 submaximal workloads and corresponding 0_2 consumption is recorded:

Workload (W)	$\dot{V}O_2$ ($L \cdot min^{-1}$)
90	1.41
120	1.79
150	2.08
180	2.58

From this information, a graph was created and a linear regression equation was determined.



Regression equation: **y = 0.011267x + 0.255**

From the $\dot{V}O_2$ peak test, $\dot{V}O_2$ peak = 4.01 L. Therefore the O_2 consumption representing 70% and 125% of $\dot{V}O_2$ peak is, (4.01 L)(.7) = 2.81 L (70% $\dot{V}O_2$ peak)
(4.01 L)(1.25) = 5.01 L (125% $\dot{V}O_2$ peak)
From regression equation (where y = O_2 consumption and x = wattage):

Estimated Wattage at 70% $\dot{V}O_2$ peak

y = 0.011267x + 0.255
2.81 = 0.011267x + 0.255
x = 2.81/0.011267 - 0.255
x = 248.88675
x = 250 W

Estimated Wattage at 125% $\dot{V}O_2$ peak

y = 0.011267x + 0.255
5.01 = 0.011267x +0 .255
x = 5.01/0.011267 - 0.255
x = 444.640133
x = 445 W

Venous Catheterization and Blood Sampling

In each of the six experimental sessions (familiarization, pre-supplementation and post-supplementation), a catheter (20 gauge, 25 mm Insyte) was inserted into the antecubital region of the subjects' left arm. The remainder of the blood sampling apparatus consisted of an injector adapter M.L. lock (Medex 7/8 inches) and a 6-inch clear pressure monitoring extension. A 0.5-mL volume, 10 I.U./ mL concentration of heparin-saline was used to maintain patency in the catheter between serial blood draws. Upon catheter insertion, the subject rested in a seated position for a minimum of ten minutes before the initial blood sample was taken. Venous blood sampling for the supramaximal exercise (125% $\dot{V}O_2$ max) consisted of four blood draws at 10 min pre exercise, immediately pre-exercise, immediately post-exercise and 10 min post exercise. During the 45-min submaximal exercise at 70% $\dot{V}O_2$ max, seven blood draws were taken at −10, 0, 15, 30, 45, +15, +30 min with (-) representing pre-exercise and (+) representing post-exercise recovery times. During the familiarization exercise sessions, the subjects were catheterized and two samples were taken for the purposes of accustoming the subject to the blood drawing procedure and reducing anxiety/concerns in subsequent sessions. The first 0.75-mL of blood was discarded from the catheter before a blood sample was taken. The volume of each blood draw was 10 mL with 5 mL extracted into a vacutainer tube containing 0.04-mL ethylenediaminetetraacetic acid (EDTA) and 5 mL into a vacutainer tube containing 0.04-mL glutathione. The total blood volume taken during the supramaximal exercise was 40 mL and 70mL during the 45-min submaximal exercise. The subjects did not receive any fluids until after the last blood sample had been taken at which time the catheter was removed and fluids were provided.

Maximum Accumulated Oxygen Deficit

The maximum accumulated oxygen deficit (MAOD) test consisted of exercise at 125% of the individual's $\dot{V}O_2$ max until exhaustion. Medbo et al. (1988) coined the term "accumulated oxygen deficit" for this test. They suggested that it was an adequate measure of anaerobic capacity. Scott et al. (1991) demonstrated a significant correlation between the MAOD test and other accepted anaerobic measures such as the Wingate test and maximal anaerobic running test. For the test, the subjects were positioned on an ergocycle (Sensormedics – Ergomedics 800S) with an air collection system to capture their expired exercise air into a 350 L wet spirometer (Collins Gasometer, Brantree, MA). The subjects performed a warm-up exercise at an intensity of 70 $W \cdot min^{-1}$ for 3 min. During this time, the expired air was diverted into the room. Subjects were instructed to try to maintain the pedaling rate at 110 rev/min and to not let it fall below 50 rev/min. The subjects were notified when the pedaling rate fell below 50 rev/min and they were permitted to continue if they could quickly raise the pedaling rate above that level. The test was terminated if the pedaling rate dropped below 50 rev/min a second time or they could not recover from the initial decrease in pedaling rate. At the beginning of the MAOD test, the power output (W) was increased to the subjects' predicted level while simultaneously the expired air was diverted to the spirometer. Exercise time was calculated from the point of an increase in power output to their predicted 125% $\dot{V}O_2$ max until the criteria for cessation had been met.

Upon completion of the test, the expired air was analyzed (Metek gas analysis system) for oxygen and carbon dioxide fractions, and oxygen consumption was corrected to STPD values (standard temperature and pressure dry). The MAOD was calculated

from standard equations that utilized the difference in measured oxygen consumption compared to earlier predicted values from the subject's linear regression equation of oxygen consumption versus power output.

Submaximal Steady-State Exercise Protocol

The submaximal exercise protocol consisted of 45 min of exercise at a level of resistance equal to 70% of the subject's $\dot{V}O_2$ max as predicted from their linear regression model. During exercise, expired air was continuously collected and analyzed for oxygen and carbon dioxide partial pressures, ventilatory volume (L/min), absolute oxygen consumption (L/min), relative oxygen consumption (mL/kg/min) and respiratory exchange ratio (RER). Serial blood draws were taken at previously described intervals with hydration provided after the final blood sample was taken.

Supplementation

Between exercise sessions 5 and 6 (Fig. 1), the subjects received a supplement of either a creatine/polycose/sucrose combination or a polycose/sucrose placebo. Supplementation packages consisted of 6 small bags: 5 bags with four vials and one bag with 3 vials. The 5 bags represented the loading phase of supplementation. The contents of a bag were consumed each day with the four vials in each bag taken at evenly spaced intervals throughout the day. The subjects were instructed to consume a vial with breakfast, with lunch, at supper and near bedtime. The 6th bag containing 3 vials was specified for days 5, 6 and 7. After the 5-day loading phase, one vial per day was consumed for 3 days to maintain creatine concentration in the body. The subjects were

instructed to dissolve the contents of each vial in 300 – 500 mL of water or juice and to stir intermittently until the supplement was consumed.

The creatine-supplemented subjects received 5g of creatine, 1.5g of polycose and 1.5g of sucrose in each loading phase vial. The placebo vials contained 6.5g of polycose and 1.5g of sucrose to match the creatine vials for volume, texture and appearance. The maintenance dose vials for the subjects receiving creatine, contained 2 g of creatine, 3g of polycose and 1.5g of sucrose. The placebo maintenance vials contained 4 g of polycose and 1.5 g of sucrose and were also matched for volume with the creatine maintenance vials. Due to different densities between creatine and polycose, total mass did not match between the creatine and placebo vials. However, by altering mass slightly, the vials were matched for volume. This maintained the integrity of the double-blind nature of the study as well as providing as much carbohydrate possible in the vials with each dose of supplementation. A loading phase protocol of 20g/day of creatine for 5 days was utilized for its proven effectiveness in raising muscle phosphocreatine (PCr) concentration and total muscle creatine (TCr) (Kreider et al, 1996, Nelson et al, 1997). In total, each subject received 106 g creatine over an 8-day period.

Polycose powder was used as a placebo because of its tasteless and texture properties that make it very similar to creatine monohydrate powder. Sucrose (1.5g) was added to all of the supplement vials to achieve a constant level of "sweetness" in the supplement to maintain the integrity of the double-blind design. The inclusion of a glucose derivative with the creatine powder combined with the consumption of the supplement with/near meals was utilized to enhance the uptake of creatine into the muscles via an insulin response (Green et al., 1996). All supplements were weighed

(Mettler AE163 electronic scale) in advance and sealed and coded by a technician who was not associated with the study. Upon completion of the entire study, the code was opened.

Biochemical Analysis

For both glucose and lactate, 50 µL of EDTA-treated blood was pipetted into labeled Eppendorf tubes containing 500 µL of 0.4M perchloric acid. This procedure immediately stops glycolysis to preserve the accuracy of the sampled blood in terms of glucose and lactate concentrations. All of the blood samples were kept in an ice water bath until they were ready for centrifugation after the exercise session. Blood samples were centrifuged for 15 min at a speed of 2800 rpm at 4 °C. The separated plasma was distributed into labeled Eppendorf tubes (growth hormone, glucose, lactate) and frozen and stored at –70 °C until analysis.

Human growth hormone concentrations were analyzed in duplicate in EDTA-treated blood using a radioimmunoassay kit (Diasorin, Saluggia, Italy). Samples (50 µL) were distributed in duplicate into tubes provided by the kit. A radioactive isotope of iodine (100 µL of I^{125}) was added to the samples. Samples were incubated for 90 min with continuous shaking at 300 rpm and then decanted and rinsed. The empty tubes were measured for radioactivity using a Cobra Auto-Gamma Counter (Packard Series 101838). All of a subject's samples were assayed at the same time to maintain consistency among individual results. A calibration curve was achieved with standards of 0.5, 1.5, 3, 8, 20 and 50 ng/mL provided in the kit. Values read by the gamma counter were converted to units of ng/mL by using a conversion formula provided by the kit manufacturer (plot of log-log coordinates).

Plasma lactate concentration was determined using the methods of Maughan (1982). This method incorporates the use of a standard lactate enzyme kit (Boehringer Mannheim) and a colorimetric analyzer (Perkin-Elmer 650-10M Fluorescence Spectrophotometer). All exercise samples were diluted further with 0.4M perchloric acid at a 2:1 ratio of acid to sample.

Glucose concentration in the EDTA/perchloric acid treated blood samples was determined using a standard glucose enzyme kit (Boehringer Mannheim) and colorimetric analysis (Gilford Stasar III Spectrophotometer) as described by Maughan (1982).

All of the samples were assayed in duplicate. An acceptable result of the analysis demonstrated a coefficient of variance (CV) between the samples of less than 10%. If the CV was greater than 10%, the samples were re-analyzed.

Statistical Analysis

All values of MAOD and metabolite values are presented as mean $\pm$ standard error of means (M $\pm$ SEM). Subject characteristics will be presented as mean $\pm$ standard deviation (M $\pm$ SD). Analysis of variance (ANOVA) was applied to the results to determine if a significant difference between means existed over time, condition (placebo vs. creatine) and the interaction of treatment and time (pre-supplementation vs. post-supplementation). A standard statistical package (SuperAnova) was utilized to perform the ANOVA tests. If significance was found ($p \leq .05$), a Newman-Keuls Post Hoc test was used to determine the mean values that were significant within the ANOVA comparison.

RESULTS

Subject Characteristics

After random assignment of the subjects to the placebo and creatine groups, the mean characteristics of each group were calculated to determine whether there was any difference between the two groups as a result of the random assignment (Table 3).

Table 3. Comparison of Subjects' Characteristics (Mean ± SD)

Subject Group	Number of Subjects (n)	O2 Consumption ($L \cdot min^{-1}$)	Body Mass (kg)	Body Fat (%)	Lean Body Mass (kg)
Placebo	10	3.7 ± 0.4	52 ± 6.8	13.4 ± 3.3	62.7 ± 5.2
Creatine	10	3.9 ± 0.5	55 ± 9.1	12.8 ± 3.8	65.0 ± 8.0

No significant differences between the two groups were found in peak $\dot{V}O_2$ consumption, body mass, percentage body fat and lean body mass. The two groups were statistically identical with respect to these variables.

Supra-Maximal Exercise

Maximum Accumulated Oxygen Deficit

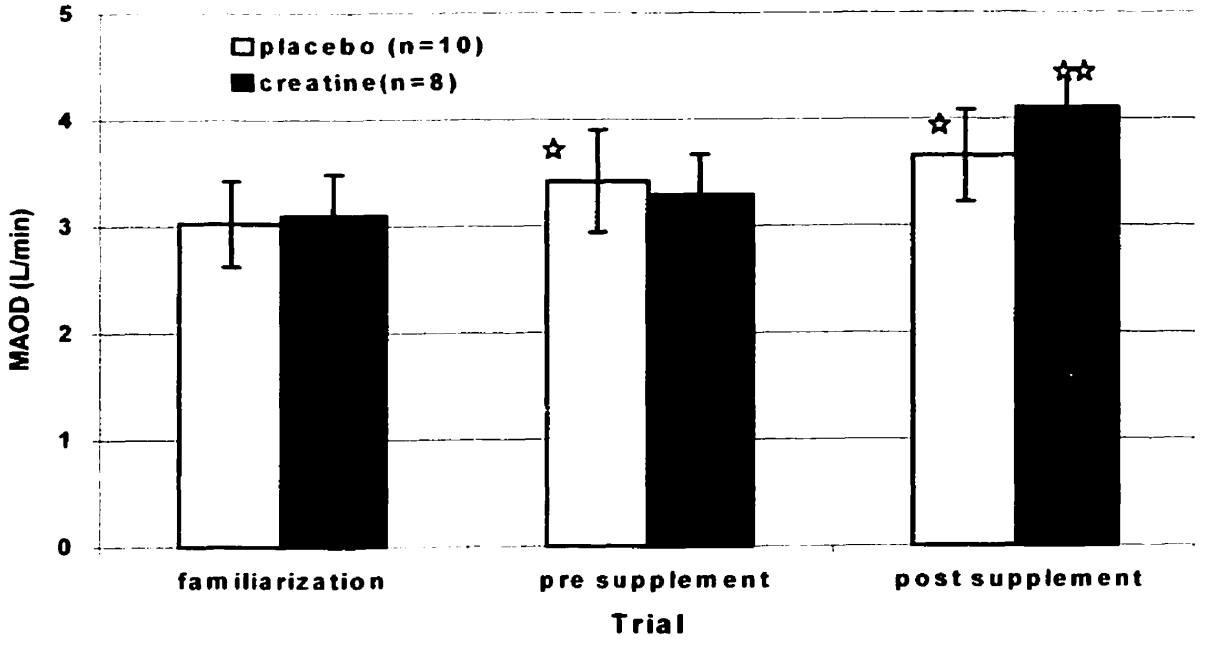
Examination of the MAOD data revealed that there might have been an existence of non-responders in the creatine supplementation group. By utilizing the MAOD results as an indirect measure of creatine muscle enhancement (thus increased MAOD performance), two of the subjects (C1 and C5) in the creatine supplementation group could be considered non-responders. The MAOD data was calculated with and without

the inclusion of the two subjects that displayed no significant increase in MAOD post-creatine supplementation. With the inclusion of these deemed "non-responders" the results were relatively unchanged with the creatine group's statistical significance being $p = 0.051$ as opposed to the $p < 0.025$ reported in Figure 2. Consequently, all data reported for the creatine group (including metabolites) was calculated with a subject number of 8.

The effects of placebo and creatine ingestion upon the maximum accumulated oxygen deficit (MAOD) and exercise time are shown in Table 4 and MAOD in Figure 2.

Upon statistical analysis of the MAOD data, a significant ($p< .025$) group vs. trial effect was observed. With respect to the placebo group, the mean MAOD values of 3.03 ± 0.40 L to 3.42 ± 0.38 L (Mean $\pm$ SEM) represent a significant increase from the familiarization trial to the pre supplementation trial. A non-significant increase in mean MAOD occurred in this group between the pre- and post-supplementation phases. These increases diminished from trial to trial in the placebo group and were attributed to a learning effect.

Figure 2.



TRIAL EFFECT OF CREATINE SUPPLEMENTATION ON MAXIMUM ACCUMULATED OXYGEN DEFICIT

☆ Denotes significant difference ($p < 0.001$) from corresponding 'familiarization' trial.
☆☆ Denotes significant difference ($p < 0.05$) from all other mean MAOD's.
All values expressed as Mean ± SEM.

Table 4. Comparison of Placebo and Creatine on Parameters of Supra-Maximal Exercise

(Mean ± SEM)

Parameter	Supplementation Phase	Placebo (n=10)	Creatine (n=8)
MAOD (L/min)	Familiarization	3.0 ± 0.4	3.1 ± 0.4
	Pre-supplement	3.4 ± 0.5 *	3.3 ± 0.4
	Post-supplement	3.7 ± 0.4 *	4.1 ± 0.4 **
Exercise Time (sec)	Pre-supplement	132.9 ± 14.4	138.2 ± 14.3
	Post-supplement	138.2 ± 14.3	155.0 ± 12.3 †

† denotes a significant difference ($p < 0.05$) from pre- to post-supplementation
* denotes significant difference ($p < 0.001$) from corresponding 'familiarization' trial.
** denotes significant difference ($p < 0.025$) from all other mean MAOD's.

The creatine group's response was similar to that of the placebo groups in that this group also demonstrated an increase (not significant) from the familiarization trial to the pre-supplementation trial – again attributed to a learning effect. After supplementation, the creatine group's performance on the MAOD test produced a mean value of 4.11 ± 0.36 L (Mean ± SEM) that was significantly higher ($p < 0.025$) than all other mean MAOD values. When comparing this value to the placebo group's post-supplementation value, a non-proportionate increase is observed in the creatine group that cannot be attributed solely to a learning curve while performing the test. A time to exhaustion of 155.0 ± 12.3 sec for the creatine group after supplementation was significantly higher ($p < 0.05$) than the placebo group's post supplementation time to exhaustion of 138.2 ± 14.3 sec.

Lactate Response

Due to a blood collection problem for a placebo group subject (P1), all blood data for the supramaximal exercise considers a subject number of 9 with the creatine group having a subject number of 8 (non-responders). The blood lactate concentrations in the placebo and creatine groups during performance of the supramaximal exercise are shown in Table 5 and Figure 3.

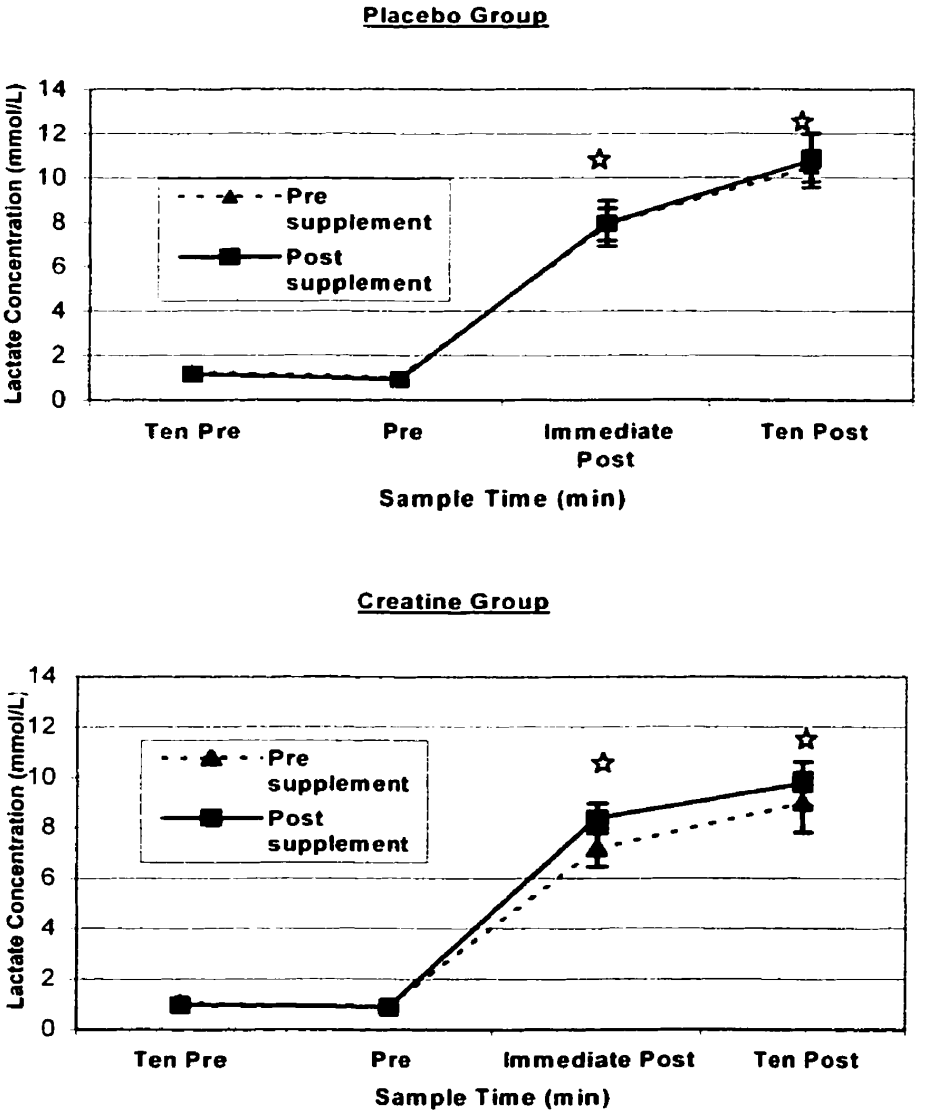
Table 5. Lactate Response During Supramaximal Exercise

Group	Treatment	10 min Pre	Pre-Ex	Post-Ex	10 min Post	Exercise Time (s)
Placebo (n = 9)	Pre-Supplement	1.26 ± 0.23	0.99 ± 0.20	7.91 * ± 1.95	10.52 * ± 2.60	132.88 ± 43.25
	Post-Supplement	1.17 ± 0.30	0.94 ± 0.28	7.95 * ± 2.75	10.80 * ± 3.59	137.76 ± 42.97
Creatine (n = 8)	Pre-Supplement	1.07 ± 0.37	0.89 ± 0.22	7.19 * ± 2.13	9.01 * ± 3.32	138.22 ± 43.01
	Post-Supplement	0.98 ± 0.23	0.89 ± 0.24	8.38 * ± 1.81	9.76 * ± 2.53	154.98 ± 36.97

* Significantly ($p < 0.001$) higher than pre-exercise values
All values expressed as Mean ± SEM and units of mmol/L

Blood lactate in the placebo groups was elevated significantly ($p < 0.001$) post-exercise (8.0 pre-supplementation and 8.0 post-supplementation) with a further increase 10 min after the completion of the exercise (10.5 pre-supplementation and 10.8 post-supplementation). A similar pattern of lactate increases was observed in the creatine groups. Creatine supplementation did not have any significant effect upon exercise-induced increases in blood lactate during supramaximal exercise. No significant interactions between the creatine and placebo groups were observed.

Figure 3.



LACTATE CONCENTRATIONS DURING 125% $\dot{V}O_2$ PEAK SUPRAMAXIMAL EXERCISE

Relationship between sample time and lactate response for supramaximal exercise before and after supplementation. ☆ A significant increase ($p < .001$) from pre-exercise concentration was seen in lactate concentrations throughout the exercise period in all groups. There was no significance ($p < .05$) between creatine and placebo groups or between pre and post supplementation. Values displayed are mean ± SEM.

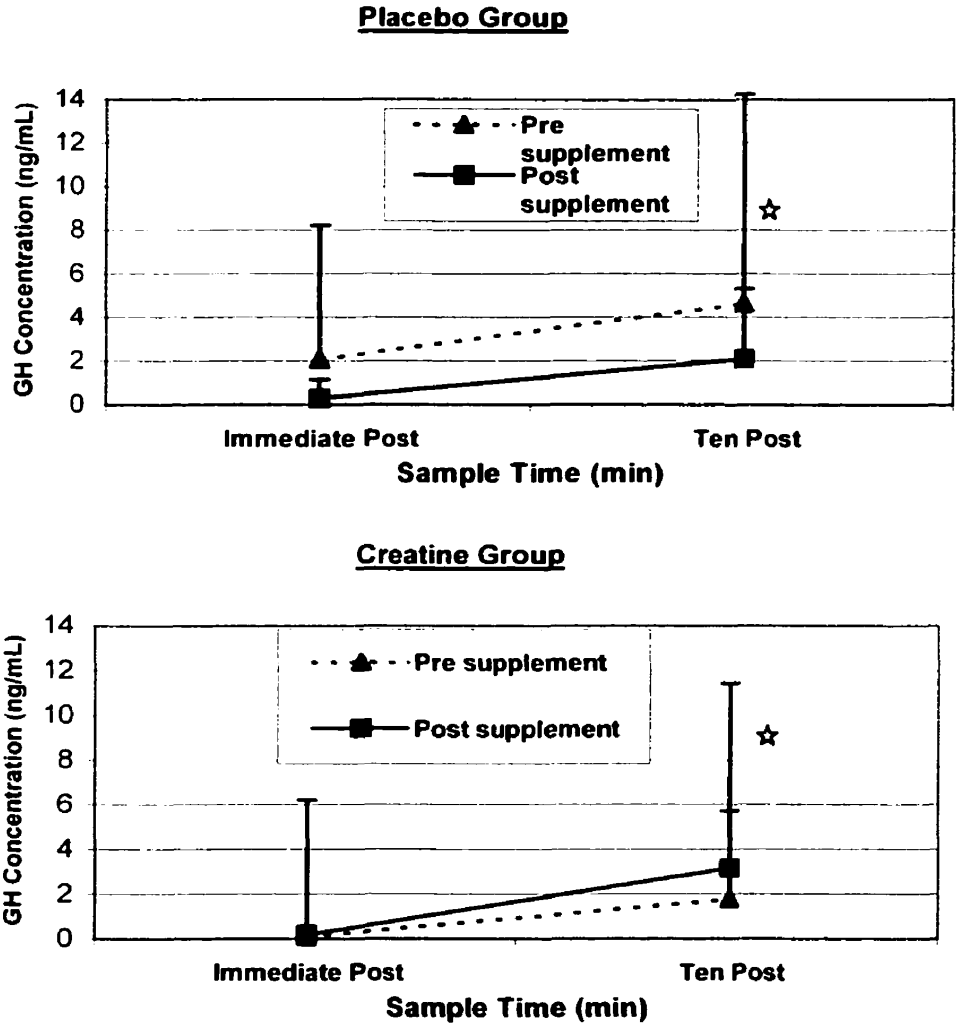
Growth Hormone Response

As expected from the strenuous exercise, GH concentration increased after the supramaximal (125% $\dot{V}O_2$ peak) exercise. To minimize the effect of large inter-group variability, values for GH were expressed as a change from baseline in Figure 4. Average baseline values of 0.61 ± 0.45 ng/mL for the creatine group and 0.46 ± 0.4 ng/mL were normal and unremarkable. GH concentrations increased after exercise but only a change of 3.0 ± 5.2 ng/mL (mean ± SEM) at 10 minutes post-exercise was significantly different ($p < .01$) from the immediate post- (Δ 0.6 ± 3.0 ng/mL, mean ± SEM) and pre-exercise concentration (baseline). No other concentration of significance existed between the groups.

Glucose Response

Glucose responses during supramaximal (125% $\dot{V}O_2$ peak) exercise are shown in Figure 5. Only plasma glucose concentration of 3.7 ± 0.9 mmol/L (mean ± SEM) recorded at 10 min post exercise were significantly different ($p < 0.05$) from pre-exercise or immediately post-exercise concentration (3.2 ± 0.7 and 3.1 ± 0.8 mmol/L, respectively). No interaction between the placebo and creatine group existed in terms of plasma glucose response.

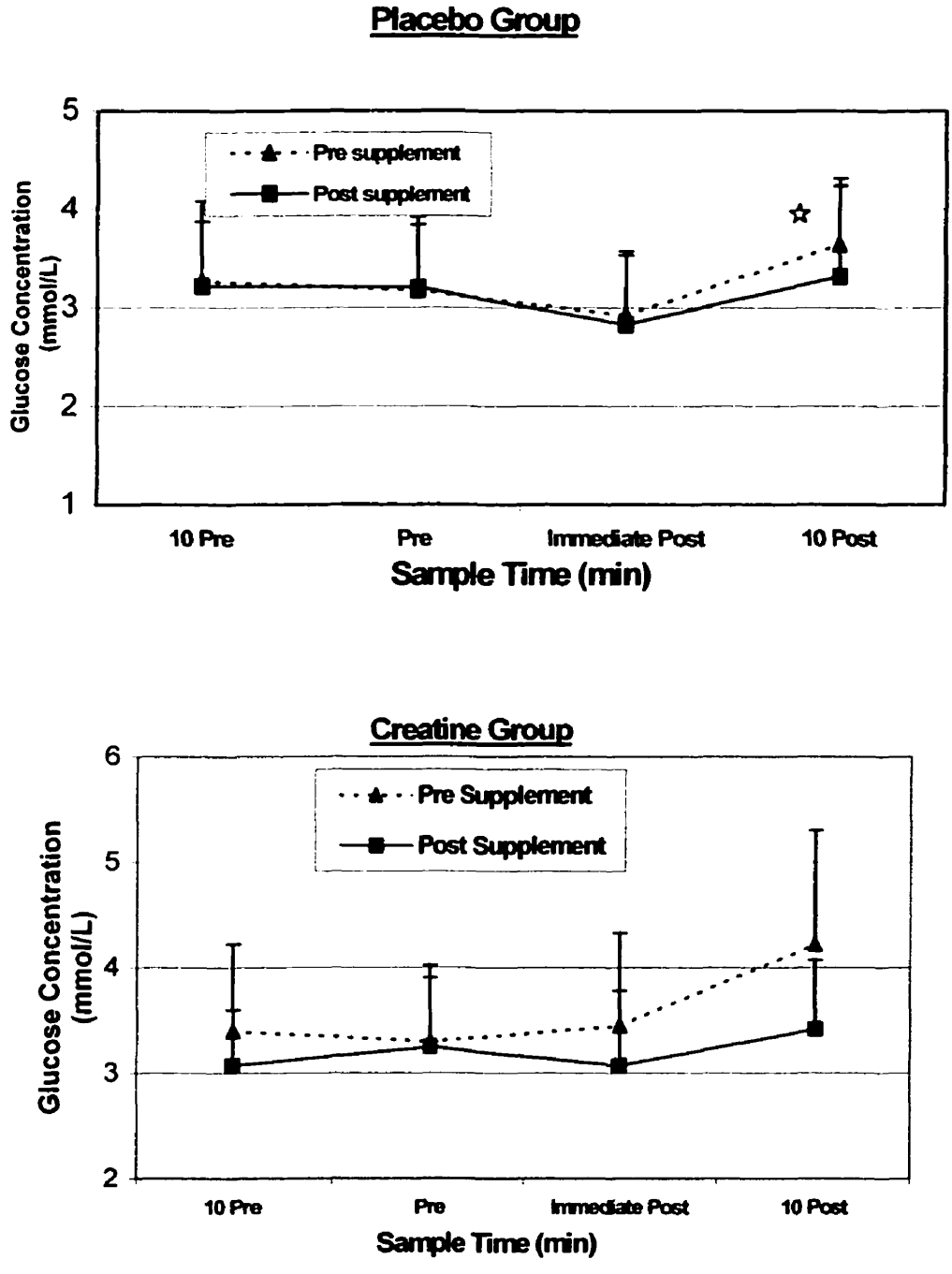
Figure 4.



GRAPH OF THE CHANGE IN GROWTH HORMONE FROM BASELINE DURING 125% $\dot{V}O_2$ PEAK EXERCISE

Relationship between sample time and GH response for supramaximal exercise before and after supplementation. ☆ Only GH concentrations at 10 minutes post-exercise were significant ($P<.05$) from baseline. Values are represented as change in GH concentration from a zero baseline (delta GH). Mean GH concentrations increased after supramaximal exercise with peak GH values observed at ten minutes post - exercise. All values are expressed as mean ± SEM.

Figure 5.



GRAPH OF GLUCOSE RESPONSE DURING 125% $\dot{V}O_2$ PEAK EXERCISE

Relationship between sample time and GL response for supramaximal exercise before and after supplementation. ☆Glucose concentration at 10 minutes post exercise were significantly different than all other times ($p < .05$). No other significant differences existed between groups and/or treatments. All values are expressed as mean ± SEM.

Prolonged Submaximal Exercise

Lactate Response

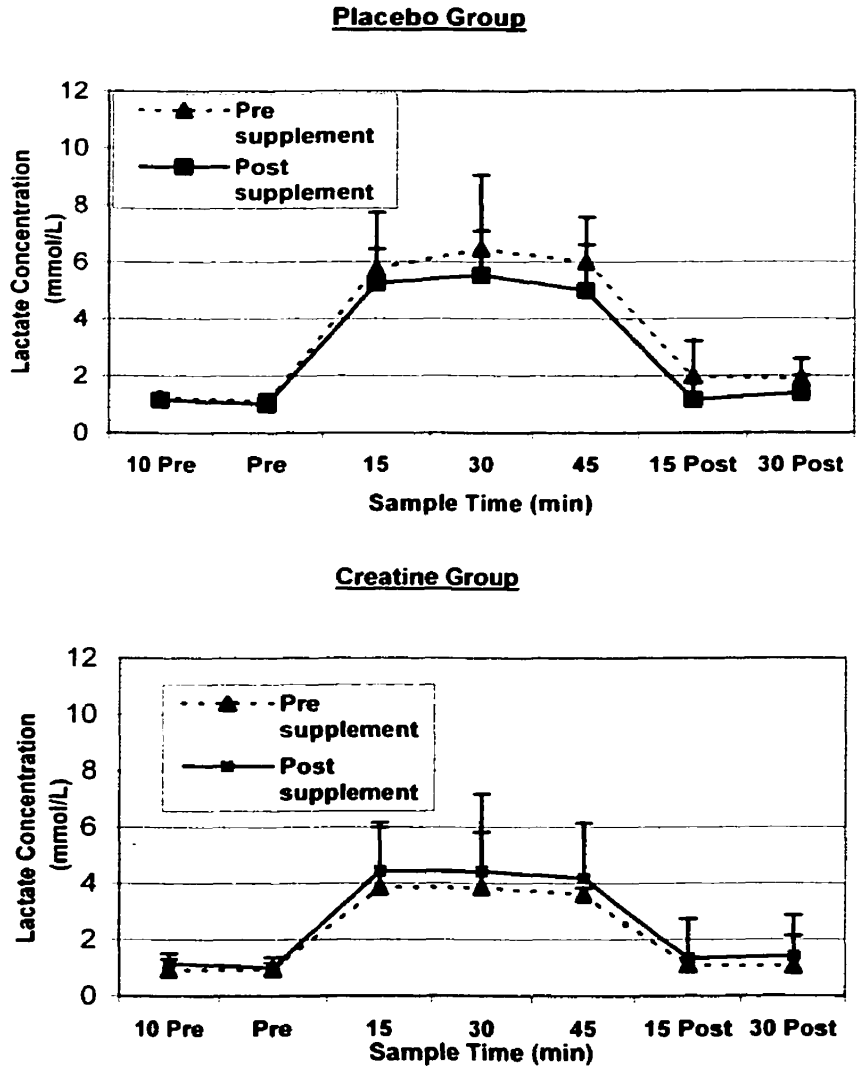
Due to blood collection problems for a subject in the placebo group during one of the trials (P10). the submaximal data was calculated with a subject number of 9 for the placebo group with the creatine group considered with a subject number of 8 (2 non-responders excluded). The blood lactate responses to the submaximal exercise are shown in Table 6 and Figure 6. Blood lactate responses demonstrated a consistent pattern over all conditions increasing significantly ($p < 0.001$) after 15 min of exercise and remaining elevated throughout the exercise period. A treatment vs. group effect existed as well ($p < .025$) with lactate means during exercise. Post hoc evaluation determined this significance to lie within the placebo group only. The interaction between sampling time, treatment and group was not significant ($p = .14$).

Table 6. Lactate Response During Submaximal Exercise

Group	Treatment	10 min Pre $mmol \cdot L^{-1}$	Pre $mmol \cdot L^{-1}$	15 min $mmol \cdot L^{-1}$	30 min $mmol \cdot L^{-1}$	45 min $mmol \cdot L^{-1}$	15 min Post $mmol \cdot L^{-1}$	30 min Post $mmol \cdot L^{-1}$
Placebo (n = 9)	Pre-Supplement	1.2 ± 0.2	1.1 ± 0.3	5.8 ± 2.0	6.5 ± 2.6	6.0 ± 1.6	2.0 ± 1.3	1.9 ± 0.7
	Post-Supplement	1.17 ± 0.22	1.0 ± 0.2	5.3 ± 1.2	5.5 ± 1.6	5.0 ± 1.6	1.2 ± 0.6	1.4 ± 0.6
Creatine (n = 8)	Pre-Supplement	0.9 ± 0.4	1.0 ± 0.4	3.9 ± 1.3	3.9 ± 1.6	3.6 ± 2.2	1.1 ± 1.1	1.1 ± 0.5
	Post-Supplement	1.2 ± 0.4	1.0 ± 0.4	4.4 ± 1.7	4.4 ± 1.4	4.2 ± 2.0	1.3 ±1.4	1.4 ± 0.7

Relationship between sample time and LA response for submaximal exercise before and after supplementation. Exercise values of lactate were significantly higher than pre- and post-exercise concentration ($p < .001$). A significant group/treatment effect also existed ($p = .025$). Post-hoc analysis determined that a significant decrease in lactate values occurred only in the placebo group from pre- to post-supplementation. All values are expressed as mean ± SEM.

Figure 6.



MEAN LACTATE RESPONSE DURING SUBMAXIMAL 70% $\dot{V}O_2$ PEAK EXERCISE

Relationship between sample time and LA response for submaximal exercise before and after supplementation. Exercise values of lactate were significantly higher than pre- and post-exercise concentration ($p < .001$). A significant group/treatment effect also existed ($p < .025$). Post-hoc analysis determined that a significant decrease in lactate values occurred only in the placebo group from pre- to post-supplementation. All values are expressed as mean $\pm$ SEM.

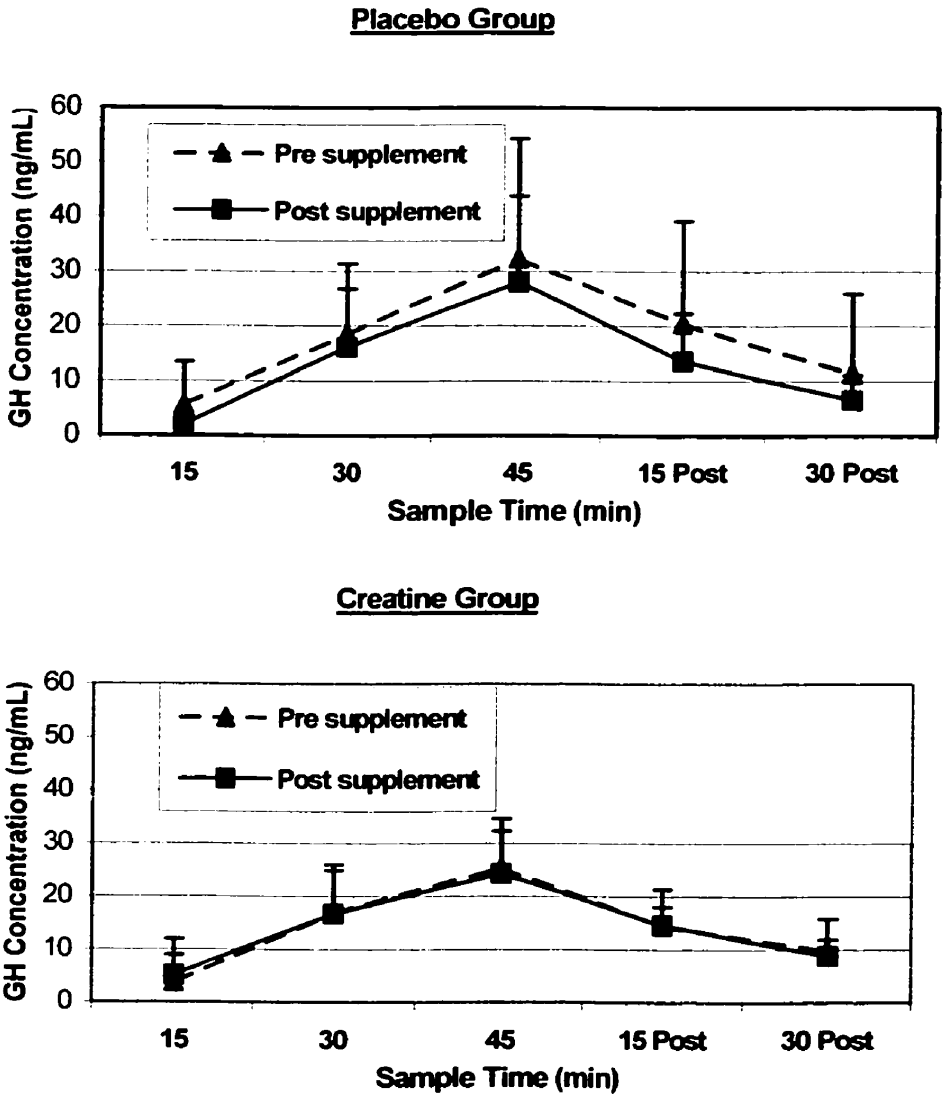
Growth Hormone Response

The results for growth hormone secretion during submaximal (70% $\dot{V}O_2$ peak) exercise followed an expected pattern of release. Again, due to a large inter-group variability, values are expressed as a change from baseline in Figure 7. Average baseline values of 0.68 ± 0.53 ng/mL for the creatine group and 1.69 ± 1.67 ng/mL were normal and unremarkable. GH increased significantly from pre-exercise concentration throughout each sampling time ($p < .05$) with the greatest concentration of 27.4 ± 14.4 ng/mL (mean ± SEM) observed at 45 min of exercise. Growth hormone concentration decreased significantly 15 and 30 min post-exercise (15.8 ± 11.0 and 9.2 ± 8.3 ng/mL, respectively). All values were significantly different from each other ($p < .05$) except for values at 30 min of exercise and 15 min post-exercise. No significant differences existed between the creatine and placebo groups

Glucose Response

The plasma glucose response to submaximal exercise (70% $\dot{V}O_2$ peak) is depicted in Figure 8. A non-significant decrease in glucose concentration from rest occurred at 15 min into exercise and then increased significantly throughout exercise and post-exercise. The plasma glucose values at 15 and 30 min post-exercise were significantly higher ($p < .001$) than all other sample times (4.4 ± 0.7 and 4.4 ± 1.0 mmol/L, respectively). Glucose concentration of 3.6 ± 0.5 mmol/L (mean + SEM) at 30 min and of 3.8 ± 0.6 mmol/L (mean + SD) at 45 min of exercise were significantly higher ($p < .05$) than values recorded pre-exercise and at 15 min of exercise. No significant differences existed between the placebo and creatine groups before or after supplementation.

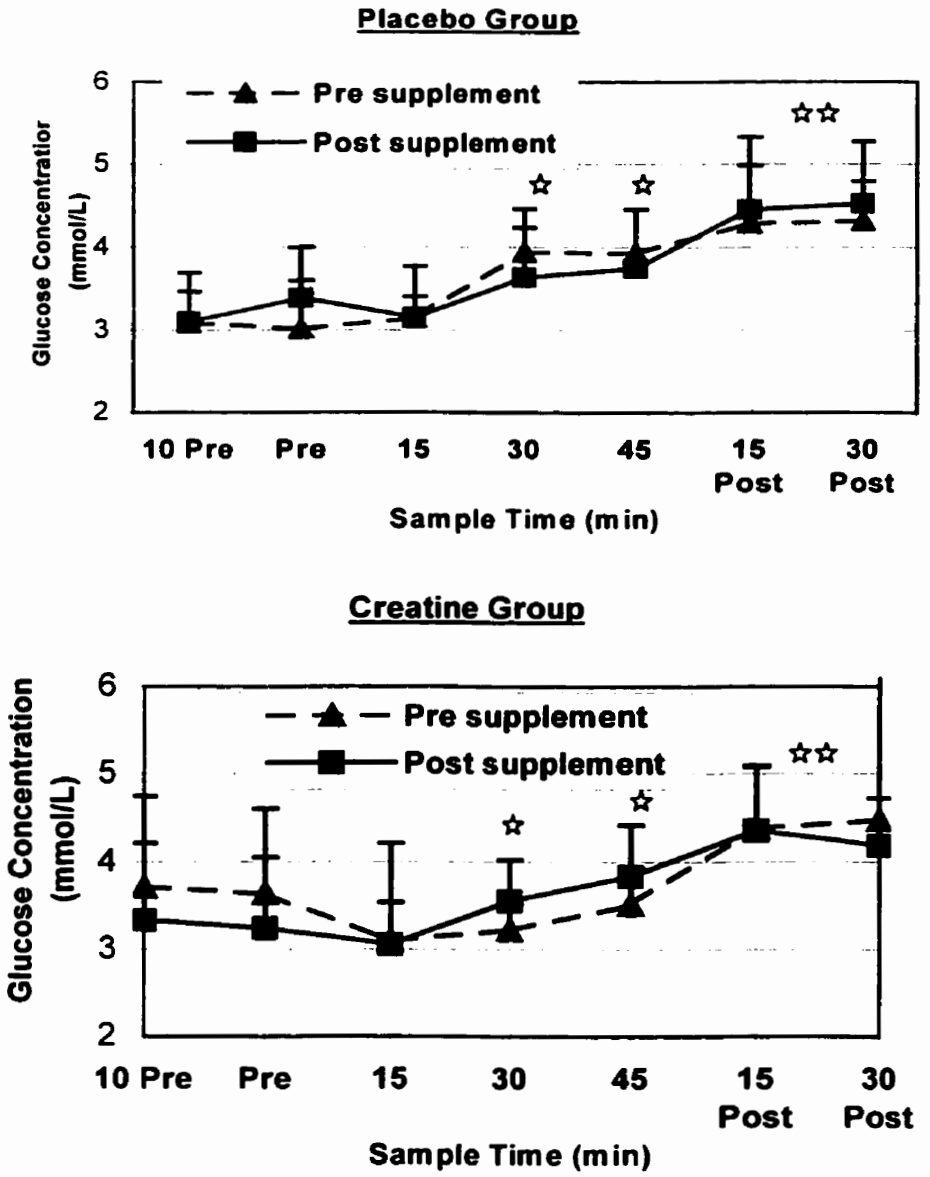
Figure 7.



CHANGES IN GH CONCENTRATION DURING SUBMAXIMAL 70% $\dot{V}O_2$ PEAK EXERCISE

Relationship between sample time and GH response for submaximal exercise before and after supplementation Values are represented as change in GH concentration from a zero baseline (delta GH). Mean GH concentrations increased significantly ($P<.05$) during submaximal exercise with peak values recorded at the end of exercise (45 min). All sample times are significantly different from each other except for 30 min and 15 min post. No other significant differences existed between the groups. All values are expressed as mean $\pm$ SEM

Figure 8.



GRAPH OF GLUCOSE RESPONSE DURING 70% $\dot{V}O_2$ PEAK EXERCISE

Relationship between sample time and GL response for submaximal exercise before and after supplementation Initially, a non-significant decrease in glucose concentration occurred (15 min) with exercise then concentration rose throughout exercise and recovery.

☆☆ Glucose concentration were significantly higher ($p < .001$) after exercise (15 and 30 minutes post) relative to all other sample times.

☆ Glucose concentration at 30 and 45 minutes were significantly higher ($p < .05$) than at 15 minutes of exercise. No further significance existed with group or treatment interactions. All values are expressed as mean $\pm$ SEM.

Discussion

The role of ergogenic aids is becoming evermore prominent in both professional and recreational exercise performance. Creatine supplementation is arguably the most popular ergogenic aid presently in use. The benefits of creatine supplementation in terms of exercise performance are well documented in the literature. However, little is known on the effect of creatine supplementation on hormone and metabolite responses during different types of exercise. The purpose of this study was to affirm that a 5-day creatine supplementation period increases the maximum accumulated oxygen deficit during short-term, supramaximal exercise with benefits equaling 6 weeks of training. As well this study attempted to elucidate the effect of creatine supplementation on the GH, LA and GL responses to aerobic and anaerobic exercise. Furthermore, if creatine supplementation increased the duration of anaerobic work performed, a duration/intensity increase in GH secretion may occur after creatine supplementation.

Supramaximal Exercise

The use of a MAOD test was implemented to determine whether a creatine loading effect was present after supplementation. Any significant differences in metabolites/hormones could only be attributed to creatine supplementation if a performance effect could be established. It has been established that not all individuals supplementing with creatine will achieve increased PCr levels high enough to elicit a significant performance increase. These individuals are deemed as non-responders. The purpose of this study was to study hormone and metabolite changes after creatine supplementation. Upon initial analysis of the MAOD data, it was apparent that two subjects (C1 and C5) did not demonstrate an increase in performance after

supplementation. It was prudent that these subjects not be included in the subsequent analysis of GH, lactate and glucose for the study was interested in changes in these blood markers only after "successful" creatine supplementation. Thus, only 8 subjects were included from the creatine group in the analysis of the data. As well, as a result of a blood collection failure (P1), only 9 subjects from the placebo group were used in the analysis of the supramaximal data.

The MAOD portion of the experimental protocol resembled that of Jacobs et al. (1997). Their work demonstrated a mean increase in MAOD from 4.04 $\pm$ 0.31 to 4.41 $\pm$ 0.34 L after a 5-day creatine supplementation period. This increase was equivalent to six weeks of anaerobic training as described by Medbo and Burgers, (1990) and provided an indirect measure of creatine muscle uptake in lieu of a muscle biopsy procedure. With a learning effect taken into consideration, an increase of approximately 0.4 L was observed in this study. The present study describes an increase in MAOD very similar to that of Jacobs' group and verifies the positive effect of creatine supplementation on anaerobic exercise and more specifically, on MAOD.

Unlike Jacobs' work, the present study found a considerable learning effect from trial to trial, especially from the familiarization trial to the pre-supplementation trial. This was expected due to the novelty of the experience for the subjects and regardless of the learning effect, a significant increase in MAOD was noted for the creatine supplementation group.

There has been some debate in the literature as to the validity of MAOD as a determinant of anaerobic capacity. Scott et al., (1991) found significant correlations with the MAOD test to Wingate power, Wingate capacity, treadmill work and 300 m time for their subjects. Maxwell and Nimmo, (1996), also reported a significant correlation with MAOD and with a maximal anaerobic running test (MART). Both investigative groups suggest that MAOD is a valid predictor of anaerobic capacity and performance.

Other investigators have analyzed the MAOD test at a more profound physiological level and produced opinions as to the accuracy of the MAOD test as a reflection of anaerobic capacity. Bangsbo (1996) contends that the assumptions used in the MAOD test are not totally accurate and this affects the validity of the MAOD test. The use of submaximal exercise concentration to determine a predicted supramaximal energy demand via linear regression is one assumption that is challenged. Bangsbo suggests that variables such as the intensity of the submaximal exercise concentration and the duration of sampling of the submaximal exercise concentration can alter the determination of the supramaximal exercise level calculation. Bangsbo also suggests that the demand for energy is not constant during intense supramaximal exercise. Unpublished data from Bangsbo demonstrated a higher energy turnover rate in the first 20 sec of supramaximal exercise as compared to the remaining part of the exercise.

Medbo (1996), challenges the contentions made by Bangsbo. This investigator suggests that the basis of Bangsbo's comments are misleading in that interpretation of the data did not allow for a adequate determination of the relationship between exercise intensity and O_2 demand. Medbo's laboratory could not report the same nonlinear response of O_2 demand at higher treadmill speeds at submaximal intensities as Bangsbo

did. Although the point-by-point refutation of Bangsbo's claims by Medbo is equally valid. it is beyond the scope of this discussion. The consensus by both of these investigative groups as well as others is that when the MAOD test is utilized in a before and after intervention design. it is a fair and consistent device to measure anaerobic energy utilization during supramaximal exercise within the same subject.

During supramaximal. anaerobic exercise. lactate accumulation increased expectedly for both the placebo and creatine groups. There were no significant differences between the creatine and placebo group. however. there was a tendency for slightly higher lactate accumulation in the creatine group after supplementation. Depending on the experimental design. the literature suggests one of two primary responses to lactate after creatine supplementation. For studies that control for work output before and after supplementation (Balsom et al., 1995). lactate accumulation decreased or remained similar after supplementation. One could derive that the same amount of work was being accomplished with lower or equal lactate accumulation. presumably from a buffering effect from the increased resynthesis of ADP to ATP. Another possible outcome involves an increase in lactate accumulation in studies involving measurements of exercise to exhaustion. Bosco et al., (1997), found that creatine supplementation extended the time to exhaustion significantly with a workload that elicited volitional fatigue at approximately 60 sec before supplementation. They reported an increase in lactate, which they attributed to a significant increase in time to exhaustion. Results from the present study demonstrate a non-significant increase in lactate during the supramaximal exercise after creatine supplementation. These results

concur with Bosco's findings in that the subjects increased their MAOD and time to exhaustion significantly with little increase in lactate production.

Glucose response for the supramaximal exercise was similar under all conditions for both the creatine and placebo groups. Glucose decreased slightly immediately after exercise and rose to concentration that were significantly higher than all other values. This increase in blood glucose 10 min into recovery is attributed to the inhibitory effects of GH and catecholamines on insulin release. Although insulin was not measured, it is assumed that the increase in GH combined with a probable increase in catecholamines inhibited insulin release and allowed an increase in blood glucose. Creatine did not have a significant effect on blood glucose concentration. It is understood that due to the intensity and short duration of the supramaximal exercise, there is a reliance on the phosphogen energy system and not blood glucose. Perhaps if creatine supplementation did have a significant effect on GH secretion, an effect on glucose response may or may not have been observed.

The GH response after supramaximal exercise was consistent with that of reported responses in previous studies. The literature suggests (Gray et al. 1993) that supramaximal exercise is not as effective at promoting a GH response as compared to prolonged, submaximal exercise. The mean plasma GH concentration after 10 min of recovery was approximately 4-5 ng/mL. This is in accordance with the observations of Kindermann et al. (1982) in response to anaerobic exercise to exhaustion lasting 1.5 min. Although the release of GH is inversely related to exercise intensity with a delay of only a few minutes during heavy work (Galbo, 1981), the sampling time for this study may

have not been optimal. An additional sampling at 15 min or 20 min post-exercise may have revealed higher GH concentration than observed at 10 min post-exercise.

A major concern with the determination of plasma GH was a rather large inter-subject variability based on the standard error of means. Despite years of research, the factors responsible for the initiation and amplitude of GH secretion are not clear. Stimuli such as heat strain, oxygen availability/oxygen demand ratio, catecholamines and a variety of others have all been implicated in the control of GH release. It is this variability that makes the identification of GH promotion factors difficult. Raynaud et al. (1983) elaborated on inter-subject variability during different types of work. Their findings mirror results from this study in that a great deal of inter-subject variability created a difficulty in observing the trends in GH response with total accuracy.

Submaximal Exercise

An aerobic component of exercise was included in the protocol primarily for the ability of submaximal exercise to produce a large GH response. Due to a blood collection failure on one exercise trial (P10), only 9 subjects are included in the submaximal data analysis. It is accepted that an exercise intensity threshold is necessary to elicit an increase in GH response. Galbo, (1981) suggested that increases in GH are discernable at exercise intensities of 10-15% of $\dot{V}O_2$ max. Viru (1985) offered that intensities of closer to 50% $\dot{V}O_2$ max were needed to induce significant increases in GH during exercise. By choosing an exercise intensity of 70% of the subjects' $\dot{V}O_2$ max, an increase in plasma GH could be expected over a 45 min exercise session and 30 min recovery. The results from the present study were consistent with the literature in that the

45-min submaximal exercise produced the greatest GH response as compared to supramaximal exercise. A lag in the plasma accumulation of GH was present in the findings of the present study with a significant increase being observed at 15 min from baseline, and substantially greater concentration at 30 min and 45 min. This observation is in accordance with the accepted lag that has been noted in the literature. Karagiorgos et al. (1979) noted a similar lag in GH accumulation with GH concentration rising throughout the 40-min exercise session at 45% $\dot{V}O_2$ max. More recently, Kanaley et al. (1997) demonstrated the same pattern with repeated aerobic exercise sessions at 70% $\dot{V}O_2$ max.

In the present study, the plasma GH levels decreased during the recovery period. At 15 min of recovery, GH concentration decreased by close to half of the 45-min exercise value. The GH concentration at 30 min of recovery were close to half of the values recorded at 15-min recovery. This is consistent with the literature that suggests that the half-life for circulating plasma GH is approximately 16 min (Lassarre et al., 1974).

In terms of a comparison between the placebo and creatine supplementation groups, there were no discernable, significant differences in GH levels before or after supplementation.

With respect to experimental design and the large intersubject variability in GH levels, a longitudinal study may have been a more appropriate choice. A cross-over design would have controlled for the large variations in GH release in the subjects. However, logistically this would have made the experiment more difficult in that the washout phase

for creatine during the cross-over portion of the experimental design would have required at least 30 days.

The research to date concerning creatine supplementation (Balsom et al. 1993; Stroud et al. 1994; Godley et al. 1997) strongly suggests that creatine does not have any beneficial effect on long-term aerobic exercise. The literature does not refer to any relationship to increased PCr stores affecting the glucose response to aerobic exercise. Factors that would attribute to increased blood glucose concentration would be elevated insulin-inhibitory hormones such as growth hormone, catecholamines and cortisol. In this study, insulin was not measured. Therefore, the observed increases in plasma GH combined with the observed increases in blood glucose would suggest that GH (and most probably other insulin-inhibitory hormones) suppressed blood insulin levels.

Statistical analysis of the lactate release during submaximal exercise demonstrated significant differences in exercise lactate values as compared to pre- and post-exercise values for all conditions. With the known relationship of lactate accumulation and exercise intensity, the increase of plasma lactate concentration was expected. In all conditions, values increased to 4-6 mmol/L and remained constant throughout the exercise period. This suggests that the exercise intensity of 70% $\dot{V}O_2$ max was sufficient enough to elicit an adequate increase in lactate production but was not intense enough to reach the lactate threshold and eventually non-compensatory increase in blood lactate that would have lead to an inevitable volitional cessation of the exercise.

Creatine supplementation has been demonstrated to increase the availability of muscle phosphogen stores with a consequential benefit to short-term, anaerobic exercise

(Harris et al., 1992, Greenhaff et al., 1993). With this increase in PCr, it is also suggested that there is an increased rate of ATP resynthesis (Casey et al. 1996) which in anaerobic exercise tends to buffer lactate accumulation by absorbing a H+ in the chemical conversion of ADP back to ATP. The exercise intensity utilized in the present study (70% $\dot{V}O_2$ max) relies primarily on glycolysis/Kreb's cycle/electron transport chain pathway to provide the ATP necessary to sustain the exercise. The fact that there was a substantial, sustained elevation in lactate throughout exercise suggests that the energy demand of the exercise was high enough to divert some of the pyruvate production through the anaerobic lactic system and to produce ATP. With creatine supplementation enhancing primarily the anaerobic alactic system, and to some extent the anaerobic lactic system, a potential opportunity arises for creatine supplementation to enhance exercise at this intensity with its partial reliance on anaerobic lactic energy production. However, as demonstrated in the literature, creatine supplementation does not enhance exercise at this intensity. This is most probably due to the fact that at this intensity, the reliance on the anaerobic lactic system is not dominant and that more efficient energy pathways can provide the necessary energy without a significant lactic acid cost. As exercise intensity increases from this point and a lactate threshold is realized, the benefits of creatine supplementation to exercise are better realized as a greater reliance on the anaerobic energy system is needed to provide ATP at a rate to sustain exercise.

The objective of the present study was to determine if creatine supplementation would affect the known responses of growth hormone, lactate and glucose during both anaerobic and aerobic exercise on a cycle ergometer. This study validated the hypothesis that creatine supplementation has a beneficial effect on short-duration, high-intensity

exercise as demonstrated by increased times to exhaustion and the improved MAOD. Creatine supplementation did not have a significant effect on any the metabolites measured during both types of exercises despite a significant increase in the anaerobic work performed. Lactate levels did not increase compared to pre-supplementation.

Recommendations for Future Research

To date, a great number of descriptive studies have been reported describing either a benefit, or lack thereof, on a variety of activities and intensities of exercise. Few studies have investigated any possible effects that creatine supplementation may have on metabolic and hormonal indices such as glucose, lactate and growth hormone. Future studies could focus on these areas:

1) Specifically with regards to GH release, future studies could employ a longitudinal crossover study to better control inter-subject variability in GH release. This inter-subject variability was apparent in this study and impacted analysis of the data.
2) Future research utilizing different methods for the provocation of GH could be studied. An example might include the use of resistance training with a consistent set/repetition structure with GH measurements obtained both before and after creatine supplementation in a double-blind, crossover study.

Reference List

1. Bangsbo, J. Oxygen Deficit: A measure of the anaerobic energy production during intense exercise. *Can J Appl Physiol.* 21(5): 350-363, 1996.

2. Bosco, C., J. Tihanyl, L. Rivalta, G. Parlato, C. Tranquilli, G. Pulvirenti, C. Foti, M. Viru, and A. Viru. Hormonal responses in strenuous jumping effort. *Jpn.J.Physiol* 46: 93-98, 1996.

3. Brenner, B. M., T. W. Meyer, and T. H. Hostetter. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N.Engl.J Med.* 307: 652-659, 1982.

4. Casey, A., D., Constantin-Teodosiu, S. Howell, E. Hultman, and P. L. Greenhaff. Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. *Am. J Physiol* 271: E31-E37, 1996.

5. Casey, A. and P. L. Greenhaff. Does dietary creatine supplementation play a role in skeletal muscle metabolism and performance? *Am.J.Clin.Nutr.* 72: 607S-617S, 2000.

6. Chanutin, A. The fate of creatine when administered to man. J Biol Chem 67, 29-37. 1926.

7. Christensen, S. E., O. L. Jorgensen, N. Moller, and H. Orskov. Characterization of growth hormone release in response to external heating. Comparison to exercise induced release. *Acta Endocrinol.(Copenh)* 107: 295-301, 1984.

8. Chwalbinska-Moneta, J., F. Krysztofiak, A. Ziemba, K. Nazar, and H. Kaciuba-Uscilko. Threshold increases in plasma growth hormone in relation to plasma catecholamine and blood lactate concentrations during progressive exercise in endurance-trained athletes. *Eur.J.Appl.Physiol* 73: 117-120, 1996.

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

10. Cooke, W. H. and W. S. Barnes. The influence of recovery duration on high-intensity exercise performance after oral creatine supplementation. *Can.J.Appl.Physiol* 22: 454-467, 1997.

11. Crist, D. M., G. T. Peake, R. B. Loftfield, J. C. Kraner, and P. A. Egan. Supplemental growth hormone alters body composition, muscle protein metabolism and serum lipids in fit adults: characterization of dose- dependent and response-recovery effects. *Mech.Ageing Dev.* 58: 191-205, 1991.

12. Cross, M. C., M. W. Radomski, W. P. VanHelder, S. G. Rhind, and R. J. Shephard. Endurance exercise with and without a thermal clamp: effects on leukocytes and leukocyte subsets. *J Appl. Physiol* 81: 822-829, 1996.

13. Culpepper, R. M. Creatine supplementation: safe as steak? [editorial]. *South. Med. J.* 91: 890-892, 1998.

14. Davies, H., N. Gazetopoulos, and C. Oliver. Ventilatory and metabolic response to graduated and prolonged exercise in normal subjects. *Clin. Sci.* 29: 443-452, 1965.

15. Dawson, B., C. Goodman, S. Lawrence, D. Preen, T. Polglaze, M. Fitzsimons, and P. Fournier. Muscle phosphocreatine repletion following single and repeated short sprint efforts. *Scand. J Med. Sci. Sports* 7: 206-213, 1997.

16. de Vries, J. H., R. J. Noorda, G. A. Voetberg, and E. A. van der Veen. Growth hormone release after the sequential use of growth hormone releasing factor and exercise. *Horm. Metab Res.* 23: 397-398, 1991.

17. Earnest, C. P., Snell, P. G., and Rodriguez, R. The effect of creatine monohydrate ingestion on anaerobic power indicies, muscular strength and body composition. Acta Physiol Scand. 153, 207-209. 1995.

18. Earnest, C. P., A. L. Almada, and T. L. Mitchell. High-performance capillary electrophoresis-pure creatine monohydrate reduces blood lipids in men and women. *Clin.Sci.(Colch.)* 91: 113-118, 1996.

19. Earnest, C. P., Almada, A., and Mitchell, T. L. Effects of creatine monohydrate ingestion on intermediate duration anaerobic treadmill running to exhaustion. J.Str.Cond.Res 11, 234-238. 1997.

20. Ekblom, B. Effects of creatine supplementation on performance. *Am.J.Sports Med.* 24: S38-S39, 1996.

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

22. Ferrari, R., C. Ceconi, A. Rodella, F. De Giuli, A. Panzali, and P. Harris. Temporal relations of the endocrine response to exercise. *Cardioscience* 2: 131-139, 1991.

23. Ferreira, M., Kreider, R., Wilson, M., and Grindstaff, P. Effects of ingesting a supplement designed to enhance creatine uptake on strength and sprint capacity. Med.Sci.Sports Exerc. 29, S146. 1997.

24. Finklestein, J. W., Roffwarg, H. P., and Boyar, R. M. Age-related change in the twenty-four hour spontaneous secretion of growth hormone. *J Clin. Endocrinol. Metab.* 35, 665-670. 1972.

25. Flanagan, D. E., M. C. Taylor, V. Parfitt, R. Mardell, P. J. Wood, and B. A. Leatherdale. Urinary growth hormone following exercise to assess growth hormone production in adults. *Clin.Endocrinol.(Oxf)* 46: 425-429, 1997.

26. Frewin, D. B., A. G. Frantz, and J. A. Downey. The effect of ambient temperature on the growth hormone and prolactin response to exercise. *Aust.J Exp.Biol Med.Sci.* 54: 97-101, 1976.

27. Friedmann, B. and W. Kindermann. Energy metabolism and regulatory hormones in women and men during endurance exercise. *Eur.J.Appl.Physiol* 59: 1-9, 1989.

28. Galbo, H. Endocrinology and metabolism in exercise. Int.J.Sports Med. 2, 203-211. 1981.

29. Galbo, H. Hormonal and metabolic adaptation to exercise. New York, Georg Thieme Verlag. 1983.

30. Godley, A., J. Yates. Effects of creatine supplementation on endurance cycling combined with short, high-intensity bouts. Med Sci Sports Exerc. 29, S251. 1997

31. Goldberg. P. and Bechtel. P. Effects of low dose creatine supplementation on strength. speed and power by male athletes. Med.Sci.Sports Exerc. 29. S251. 1995.

31. Gordon. S. E., W. J. Kraemer. N. H. Vos. J. M. Lynch. and H. G. Knuttgen. Effect of acid-base balance on the growth hormone response to acute high-intensity cycle exercise. *J.Appl.Physiol* 76: 821-829. 1994.

32. Gray. A. B., R. D. Telford. and M. J. Weidemann. Endocrine response to intense interval exercise. *Eur.J.Appl.Physiol* 66: 366-371. 1993.

33. Green. A. L., E. Hultman. I. A. Macdonald. D. A. Sewell. and P. L. Greenhaff. Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. *Am.J.Physiol* 271: E821-E826. 1996.

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

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

36. Greenhaff, P. L. Creatine supplementation: recent developments [editorial]. *Br.J.Sports Med.* 30: 276-277, 1996.

37. Greenhaff, P.L. Renal dysfunction accompanying oral creatine supplements [letter: comment]. *Lancet* 352: 233-234, 1998.

38. Grindstaff, P. D., R. Kreider, R. Bishop, M. Wilson, L. Wood, C. Alexander, and A. Almada. Effects of creatine supplementation on repetitive sprint performance and body composition in competitive swimmers. *Int.J Sport Nutr.* 7: 330-346, 1997.

39. Hakkinen, K. and A. Pakarinen. Acute hormonal responses to two different fatiguing heavy-resistance protocols in male athletes. *J Appl.Physiol* 74: 882-887, 1993.

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

41. Hartman, M. L., J. D. Veldhuis, and M. O. Thorner. Normal control of growth hormone secretion. *Horm.Res.* 40: 37-47, 1993.

42. Hindmarsh, P. C. and P. G. Swift. An assessment of growth hormone provocation tests. *Arch.Dis.Child* 72: 362-367, 1995.

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

44. Jacobs, I., S. Bleue, and J. Goodman. Creatine ingestion increases anaerobic capacity and maximum accumulated oxygen deficit. *Can.J Appl.Physiol* 22: 231-243, 1997.

45. Jacobs, I. Dietary creatine monohydrate supplementation. *Can.J.Appl.Physiol* 24: 503-514, 1999.

46. Jones, A. M., T. Atter, and K. P. Georg. Oral creatine supplementation improves multiple sprint performance in elite ice-hockey players. *J.Sports Med.Phys.Fitness* 39: 189-196, 1999.

47. Kaciuba-Uscilko, H., B. Kruk, M. Szczpaczewska, B. Opaszowski, E. Stupnicka, B. Bicz, and K. Nazar. Metabolic, body temperature and hormonal responses to repeated periods of prolonged cycle-ergometer exercise in men. *Eur.J.Appl.Physiol* 64: 26-31, 1992.

48. Kanaley, J. A., J. Y. Weltman, J. D. Veldhuis, A. D. Rogol, M. L. Hartman, and A. Weltman. Human growth hormone response to repeated bouts of aerobic exercise. *J Appl.Physiol* 83: 1756-1761, 1997.

49. Karagiorgos, A., J. F. Garcia, and G. A. Brooks. Growth hormone response to continuous and intermittent exercise. *Med.Sci.Sports* 11: 302-307, 1979.

50. Kern, W., B. Perras, R. Wodick, H. L. Fehm, and J. Born. Hormonal secretion during nighttime sleep indicating stress of daytime exercise. *J Appl.Physiol* 79: 1461-1468, 1995.

51. Kindermann, W., A. Schnabel, W. M. Schmitt, G. Biro, J. Cassens, and F. Weber. Catecholamines, growth hormone, cortisol, insulin, and sex hormones in anaerobic and aerobic exercise. *Eur.J Appl.Physiol* 49: 389-399, 1982.

52. Kirskey, K., Warren, B., and Stone, M. The effects of six weeks of creatine monohydrate supplementation in male and female track athletes. Med.Sci.Sports Exerc. 29, S145. 1997.

53. Kjaer, M., J. Bangsbo, G. Lortie, and H. Galbo. Hormonal response to exercise in humans: influence of hypoxia and physical training. *Am.J.Physiol* 254: R197-R203, 1988.

54. Kracier, J., Sheppard, M. S., and Luke, J. Effect of withdrawl of somatostatin and growth hormone (GH) - releasing factor on GH release in vitro. endocrinol. 122, 1810-1815. 1988.

55. Kreider, R. B., R. Klesges, K. Harmon, P. Grindstaff, L. Ramsey, D. Bullen, L. Wood, Y. Li, and A. Almada. Effects of ingesting supplements designed to promote lean tissue accretion on body composition during resistance training. *Int.J Sport Nutr.* 6: 234-246, 1996.

56. Kreider, R. B., Ferreira, M., and Wilson, M. Effects of creatine supplementation on body composition, strength and sprint performance. Med.Sci.Sports Exerc. 30, 73-82. 1998.

57. Kurosawa, Y., Iwane, H., and Hamaoka, T. Effects of oral creatine supplementation on high and low intensity grip exercise performance. Med.Sci.Sports Exerc. 29, S251. 1997.

58. Lassarre, C., F. Girard, J. Durand, and J. Raynaud. Kinetics of human growth hormone during submaximal exercise. *J Appl.Physiol* 37: 826-830, 1974.

59. Leenders, N. and Lesniewski, L. Dietary creatine supplementation and swimming performance. Overtraining and Overreaching in Sport Conference Abstracts 1, 80. 1996.

60. Lemon, P, Boska, M., and Bredle, D. Effect of oral creatine supplementation on energetics during repeated maximal muscle contractions. Med.Sci.Sports Exerc. 27, S204. 1995.

61. Leung, D. W., S. A. Spencer, G. Cachianes, R. G. Hammonds, C. Collins, W. J. Henzel, R. Barnard, M. J. Waters, and W. I. Wood. Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature* 330: 537-543, 1987.

62. Luger, A., P. A. Deuster, P. W. Gold, D. L. Loriaux, and G. P. Chrousos. Hormonal responses to the stress of exercise. *Adv.Exp.Med.Biol.* 245: 273-280, 1988.

63. Martha, P. M., Jr., A. D. Rogol, J. D. Veldhuis, J. R. Kerrigan, D. W. Goodman, and R. M. Blizzard. Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. *J Clin.Endocrinol.Metab* 69: 563-570, 1989.

64. Maughan, R. J. A simple, rapid method for the determination of glucose, lactate, pyruvate, alanine, 3-hydroxybutyrate and acetoacetate on a single 20- mul blood sample. *Clin.Chim.Acta* 122: 231-240, 1982.

65. Maxwell, N.S., M. Nimmo. Anaerobic capacity: a maximal anaerobic running test versus the maximal accumulated oxygen deficit. *Can J Appl Physiol.* 21(1): 35-47, 1996.

66. Medbo, J. I., A. C. Mohn, I. Tabata, R. Bahr, O. Vaage, and O. M. Sejersted. Anaerobic capacity determined by maximal accumulated O2 deficit. *J Appl.Physiol* 64: 50-60, 1988.

67. Medbo, J.I. Medbo responds to Bangsbo's paper. *Can J Appl Physiol.* 21(5): 364-369, 1996.

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

69. Nevill, M. E., D. J. Holmyard, G. M. Hall, P. Allsop, A. van Oosterhout, J. M. Burrin, and A. M. Nevill. Growth hormone responses to treadmill sprinting in sp. *Eur.J.Appl.Physiol* 72: 460-467, 1996.

70. Odland, L. M., MacDougall, J. D., and Tarnopolsky, M. A. The effect of oral Cr supplementation on muscle (PCr) and power output during a short-term maximal cycling task. Med.Sci.Sports Exerc. 26(suppl. 5), S23. 1994.

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

72. Poortmans, J. R., H. Auquier, V. Renaut, A. Durussel, M. Saugy, and G. R. Brisson. Effect of short-term creatine supplementation on renal responses in men. *Eur.J Appl.Physiol* 76: 566-567, 1997.

73. Poortmans, J. R. and M. Francaux. Long-term oral creatine supplementation does not impair renal function in healthy athletes [see comments]. *Med.Sci.Sports Exerc.* 31: 1108-1110, 1999.

74. Postel-Vinay, M. C. and P. A. Kelly. Growth hormone receptor signalling. *Baillieres Clin.Endocrinol.Metab* 10: 323-336, 1996.

75. Prevost, M. C., A. G. Nelson, and G. S. Morris. Creatine supplementation enhances intermittent work performance. *Res.Q.Exerc.Sport* 68: 233-240, 1997.

76. Radomski, M. W., M. Cross, and A. Buguet. Exercise-induced hyperthermia and hormonal responses to exercise. *Can.J.Physiol Pharmacol.* 76: 547-552, 1998.

77. Raynaud, J., A. Capderou, J. P. Martineaud, J. Bordachar, and J. Durand. Intersubject viability in growth hormone time course during different types of work. *J Appl.Physiol* 55: 1682-1687, 1983.

78. Robinson, T. M., D. A. Sewell, E. Hultman, and P. L. Greenhaff. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. *J.Appl.Physiol* 87: 598-604, 1999.

79. Robinson, T. M., D. A. Sewell, A. Casey, G. Steenge, and P. L. Greenhaff. Dietary creatine supplementation does not affect some haematological indices, or indices of muscle damage and hepatic and renal function [In Process Citation]. *Br.J.Sports Med.* 34: 284-288, 2000.

80. Ruden, T. and Parcell, A. Effects of oral creatine supplementation on energetics during repeated maximal muscle contractions. Med.Sci.Sports Exerc. 28, S81. 1996.

81. Scott, C. B., F. B. Roby, T. G. Lohman, and J. C. Bunt. The maximally accumulated oxygen deficit as an indicator of anaerobic capacity. *Med.Sci.Sports Exerc.* 23: 618-624, 1991.

82. Silverman, H. G. and R. S. Mazzeo. Hormonal responses to maximal and submaximal exercise in trained and untrained men of various ages. *J.Gerontol.A Biol.Sci.Med.Sci.* 51: B30-B37, 1996.

83. Sipila, I., Rapola, J., and Simmell, O. Supplementary creatine as a treatment for gyrate atrophy of the choroid and retina. N.Engl.J Med. 304, 867-870. 1981.

84. Smith, S. A., S. J. Montain, R. P. Matott, G. P. Zientara, F. A. Jolesz, and R. A. Fielding. Effects of creatine supplementation on the energy cost of muscle contraction: a 31P-MRS study. *J.Appl.Physiol* 87: 116-123, 1999.

85. Snow, R. J., M. J. McKenna, S. E. Selig, J. Kemp, C. G. Stathis, and S. Zhao. Effect of creatine supplementation on sprint exercise performance and muscle metabolism. *J Appl. Physiol* 84: 1667-1673, 1998.

86. Steenge, G., Lambourne, J., and Casey, A. Stimulatory effect of insulin on creatine accumulation in human skeletal muscle. Am.J.Physiol 275, E974-E979. 1998.

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

88. Sutton, J. and L. Lazarus. Growth hormone in exercise: comparison of physiological and pharmacological stimuli. *J Appl. Physiol* 41: 523-527, 1976.

89. Sutton, J. R., N. L. Jones, and C. J. Toews. Growth hormone secretion in acid-base alterations at rest and during exercise. *Clin.Sci.Mol.Med.* 50: 241-247, 1976.

90. Suzuki, K., M. Totsuka, S. Nakaji, M. Yamada, S. Kudoh, Q. Liu, K. Sugawara, K. Yamaya, and K. Sato. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. *J.Appl.Physiol* 87: 1360-1367, 1999.

91. Tarnopolsky, L. J., J. D. MacDougall, S. A. Atkinson, M. A. Tarnopolsky, and J. R. Sutton. Gender differences in substrate for endurance exercise. *J.Appl.Physiol* 68: 302-308, 1990.

92. Terrillion, K. A., F. W. Kolkhorst, F. A. Dolgener, and S. J. Joslyn. The effect of creatine supplementation on two 700-m maximal running bouts. *Int.J.Sport Nutr.* 7: 138-143, 1997.

93. Thompson, C. H., G. J. Kemp, A. L. Sanderson, R. M. Dixon, P. Styles, D. J. Taylor, and G. K. Radda. Effect of creatine on aerobic and anaerobic metabolism in skeletal muscle in swimmers. *Br.J.Sports Med.* 30: 222-225, 1996.

94. Urbanski, R. L., W. J. Vincent, and B. B. Yaspelkis, III. Creatine supplementation differentially affects maximal isometric strength and time to fatigue in large and small muscle groups. *Int.J.Sport Nutr.* 9: 136-145, 1999.

95. van Leemputte, M., K. Vandenberghe, and P. Hespel. Shortening of muscle relaxation time after creatine loading. *J.Appl.Physiol* 86: 840-844, 1999.

96. Vanakoski, J., V. Kosunen, E. Meririnne, and T. Seppala. Creatine and caffeine in anaerobic and aerobic exercise: effects on physical performance and pharmacokinetic considerations. *Int.J.Clin.Pharmacol.Ther.* 36: 258-262, 1998.

97. Vandenberghe, K., N. Gillis, M. van Leemputte, P. Van Hecke, F. Vanstapel, and P. Hespel. Caffeine counteracts the ergogenic action of muscle creatine loading. *J.Appl.Physiol* 80: 452-457, 1996.

98. Vandenberghe, K., M. Goris, P. Van Hecke, M. van Leemputte, L. Vangerven, and P. Hespel. Long-term creatine intake is beneficial to muscle performance during resistance training. *J.Appl.Physiol* 83: 2055-2063, 1997.

99. Vandenberghe, K., P. Van Hecke, M. van Leemputte, F. Vanstapel, and P. Hespel. Phosphocreatine resynthesis is not affected by creatine loading. *Med.Sci.Sports Exerc.* 31: 236-242, 1999.

100. VanHelder, W. P., R. C. Goode, and M. W. Radomski. Effect of anaerobic and aerobic exercise of equal duration and work expenditure on plasma growth hormone concentration. *Eur.J Appl.Physiol* 52: 255-257, 1984a.

101. VanHelder, W. P., M. W. Radomski, and R. C. Goode. Growth hormone responses during intermittent weight lifting exercise in men. *Eur.J Appl.Physiol* 53: 31-34, 1984b.

102. VanHelder, W. P., M. W. Radomski, R. C. Goode, and K. Casey. Hormonal and metabolic response to three types of exercise of equal duration and external work output. *Eur.J Appl.Physiol* 54: 337-342, 1985.

103. VanHelder, W. P., K. Casey, R. C. Goode, and W. M. Radomski. Growth hormone regulation in two types of aerobic exercise of equal oxygen uptake. *Eur.J Appl.Physiol* 55: 236-239, 1986.

104. VanHelder, W. P., K. Casey, and M. W. Radomski. Regulation of growth hormone during exercise by oxygen demand and availability. *Eur.J Appl.Physiol* 56: 628-632, 1987.

105. Viru, A. Hormones in muscular activity. Boca Raton, CRC Press. 1985.

106. Viru, M., E. Jansson, A. Viru, and C. J. Sundberg. Effect of restricted blood flow on exercise-induced hormone changes in healthy men. *Eur.J.Appl.Physiol* 77: 517-522, 1998.

107. Vittone, J., M. R. Blackman, J. Busby-Whitehead, C. Tsiao, K. J. Stewart, J. Tobin, T. Stevens, M. F. Bellantoni, M. A. Rogers, G. Baumann, J. Roth, S. M. Harman, and R. G. Spencer. Effects of single nightly injections of growth hormone-releasing hormone (GHRH 1-29) in healthy elderly men. *Metabolism* 46: 89-96, 1997.

108. Volek, J. S., W. J. Kraemer, J. A. Bush, M. Boetes, T. Incledon, K. L. Clark, and J. M. Lynch. Creatine supplementation enhances muscular performance during high- intensity resistance exercise. *J.Am.Diet.Assoc.* 97: 765-770, 1997.

109. Volek, J. S., N. D. Duncan, S. A. Mazzetti, R. S. Staron, M. Putukian, A. L. Gomez, D. R. Pearson, W. J. Fink, and W. J. Kraemer. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med.Sci.Sports Exerc.* 31: 1147-1156, 1999.

110. Vorobiev, D. V., E. G. Vetrova, I. M. Larina, I. A. Popova, and A. I. Grigoriev. Energy substrates, hormone responses and glucocorticoid binding in lymphocytes during intense physical exercise in humans following phosphocreatine administration. *Eur.J.Appl.Physiol* 74: 534-540, 1996.

111. Walker, J. B. Creatine: biosynthesis, regulation, and function. *Adv.Enzymol.Relat Areas Mol.Biol* 50: 177-242, 1979.

112. Weeke, J. and H. J. Gundersen. The effect of heating and central cooling on serum TSH, GH, and norepinephrine in resting normal man. *Acta Physiol Scand.* 117: 33-39, 1983.

113. Weltman, A., J. Y. Weltman, C. J. Womack, S. E. Davis, J. L. Blumer, G. A. Gaesser, and M. L. Hartman. Exercise training decreases the growth hormone (GH) response to acute constant-load exercise. *Med.Sci.Sports Exerc.* 29: 669-676, 1997.

114. Yarasheski, K. E. Growth hormone effects on metabolism, body composition, muscle mass, and strength. *Exerc.Sport Sci.Rev.* 22: 285-312, 1994.

Appendix A

Subject Data

Date: _____

Name: _____

Age (years): _____

Height (cm): _____

Mass (kg): _____

Seat Height (cm): _____

VO_2 max (L/min): _____

VO_2 max (mL/kg/min): _____

HR max (bpm): _____

Final Workload: _____

Skinfolds

Triceps _____

Subscapularis_____

Suprailiac_____

Abdominal_____

Front Thigh_____

SOS = _____

% Body Fat = _____

Appendix B

Volunteer Consent Form Protocol

Title: The Effect of Creatine Supplementation on the Release of Growth Hormone During and After Aerobic Exercise

Principal Investigator: M. Radomski

Co-Investigators: J. Sandison, I. Jacobs

1. I, _____ of _____ (address and telephone number) hereby volunteer to participate as a test subject in a DCIEM experiment (protocol #L-216) to study the effects of the ingestion of creatine on the release of growth hormone during aerobic activity. I have read the attached protocol and description of risks, and have been informed to my satisfaction of all details of the procedures. I have had the opportunity to discuss any questions I may have with a DCIEM physician.

2. I am aware that the experiment consists of seven (7) visits to the laboratory, each visit consisting of exercise testing and lasting one to 2.5 hours in duration for a total of approximately 10 hours. I will be asked to do a VO_{2peak} test lasting about 10 minutes, one steady state exercise test at varied workloads and three supramaximal tests lasting 2-3 minutes on the bicycle ergometer; I will exercise to voluntary exhaustion on these tests. I will also perform three (3) – 45 minute aerobic tests at 70% VO_{2peak}. I am aware that the test procedures will include the insertion of a blood catheter of the purpose of attaining blood samples before, during and after exercise. I have been made aware of the discomfort involved with this procedure and the associated risks. I will sign a separate invasive medical procedures consent form consenting to those procedures.

3. I am aware that I will be asked to ingest both creatine and glucose or glucose only (dissolved in water) for 5 days between visits 4 and 5. I agree to the double blind fashion of the experiment and understand that neither the presiding scientist nor I will be aware at the time of testing whether I have ingested creatine or placebo. I am aware that there are inherent, unknown and unquantifiable risks associated with any scientific research and acknowledge that all know risks have been explained to me to my satisfaction.

4. I agree not to consume caffeine for 24 hours before the experiment and not to consume any breakfast on the morning of the experimental session except for the breakfast provided for me. I agree not to consume alcohol and not to engage in physical exercise for 48 hours before each testing session.

5. I am aware that exercise has infrequently resulted in heart attacks and that emergency resuscitation equipment and medications will be available at all times. Furthermore, I understand that qualified technicians and physiologists will monitor me at all times. I understand that no other experimental technique will be used requiring penetration into any body orifice of direct penetration through the skin, other that the blood catheter.

6. I am aware of the risks associated with hard exercise, such as a risk of less than 1/10,000 of a heart attack, and muscle strain and/or joint sprains. I understand that a medical interview will be required before any participation begins. Before I begin any experimental sessions, I must inform the investigators of any changes to my medical status. This information will include any medications I have taken and medical or dental treatment I have received since signing this consent.

7. I am aware that I agree that I must not donate blood within 30 days of any part of this experiment. I am also aware of the requirement to sign a separate consent form for invasive medical procedures. In the highly unlikely event that I become incapacitated during my participation, I hereby consent to whatever emergency medical intervention deemed necessary by the attending medical personnel. I also agree that I will go with the investigator to seek emergency medical attention if either the investigator or I consider that it is required.

8. I acknowledge that I have read this form and I understand that my consent is voluntary and has been given under circumstances in which I can exercise free power of choice. I have been informed that I may, at any time, revoke my consent and withdraw from the experiment without adverse consequences, and that the investigators or the physician may terminate my involvement in the experiment, regardless of my wishes.

Signature _____

Print Name _____

Witness _____ Date_____

Subject fit to participate as assessed by physician_____

(document continues on reverse)

For military personnel on permanent strength at CFEME:

Approval in principle by Commanding Officer is given in Memorandum 3700-1 (CO CFEME), 18 Aug 94; however, members must still obtain their Sector Head's signature designating approval to participate in this particular experiment. CF personnel are considered to b on duty for disciplinary, administrative and Pension Act purposes during their participation in this experiment.

For other military personnel:

All other military personnel must obtain their Commanding Officer's signature designating approval to participate in this experiment.

For civilian personnel at DCIEM:

Signature of your Sector Head is required designating approval to participate in this experiment.

Sector Head's/Commanding Officer's Signature: _____

CO's Unit _____

Principal Investigator: _____

Appendix C

Invasive Procedures Consent Form

Protocol # L-216

Principal Investigator: M. Radomski

Co-Investigators: J. Sandison, I. Jacobs

Title: The Effect of Creatine Supplementation on the Release of Growth Hormone During and After Aerobic Exercise.

(Subject: Initial beside the procedure indicating your agreement)

1._____***Venous Catheterization and Venipuncture***: A small needle is used to pierce the skin overlying the antecubital (around the elbow area of the forearm) vein and a plastic tube is left in place in order that multiple blood samples can be taken (for the catheterization procedure only). Either a physician or a properly qualified and physician authorized technician performs the insertion. The blood sampled in any one experiment will be replaced over the course of approximately 3 days. Occasionally, fainting due to nervous reflexes may occur during the procedure. Other complications may include infection of the wound site and leaking of blood into the surrounding tissue (bruising). Extremely rarely, loss of a broken of the catheter or injection of a quantity of air (in excess of 50 mL) into the circulation may cause severe, life threatening complications.

I _____ hereby consent to the procedures above. The Investigator has explained these procedures and their complications to my satisfaction, and I have had the opportunity to ask questions both of the Investigator and of a physician.

Signature: _____ Date:_____

Print Name: _____ Date: _____

Witness: _____ Date: _____

Appendix D

MAOD Test – Data Sheet

Subject's Name: _____

Exercise Run: Familiarization Control Post-Supplementation

Room Air Pressure: _____

Start Measurement –Tissot One: _____

End Measurement –Tissot One: _____

Correction Factor – Tissot One: 324.4 cc/mm

Total Volume – Tissot One: _____

Ambient Temperature –Tissot One: _____

Start Measurement – Tissot Two: _____

End Measurement – Tissot Two: _____

Correction Factor – Tissot One: 133.2 cc/mm

Total Volume – Tissot One: _____

Ambient Temperature – Tissot Two: _____

Total Exercise Time: _____ sec

Final Heart Rate: _____

Appendix E GH Raw Data Pre-Supplement

Group	CA1	CA2	CA3	CA4	CB1	CB2	CB3	CB4	CB5	CB6	CB7
	10 min pre	pre	post	10min post	10 min pre	pre	15 min	30 min	45 min	15 min post	30 min post
C1	0.270	0.138	0.121	0.323	0.032	0.038	0.395	9.152	18.698	15.762	16.043
C2	4.641	2.787	1.799	4.057	3.287	5.428	11.946	18.252	18.066	9.735	6.507
C3	0.220	0.319	0.767	5.742	0.336	0.695	17.265	29.944	28.230	11.785	7.105
C4	0.431	0.243	0.578	0.198	0.165	0.146	4.458	30.996	39.137	22.490	19.611
C5	0.028	0.034	0.249	9.162	0.036	0.073	1.307	17.282	33.174	14.537	6.480
C6	0.152	0.136	0.143	0.739	0.109	0.076	0.147	1.460	10.606	7.910	4.591
C7	0.135	0.107	0.107	0.171	0.110	0.137	1.786	13.236	19.778	7.563	5.232
C8	0.104	0.070	0.084	0.425	0.035	0.033	0.594	12.317	28.225	26.792	16.506
C9	0.100	0.088	0.136	0.642	0.072	0.099	2.718	23.687	35.167	15.548	7.437
C10	0.111	0.091	0.120	1.453	0.078	0.092	3.327	16.989	27.046	18.613	13.992
P1	x	x	x	x	0.051	0.053	2.701	36.516	35.702	25.526	11.458
P2	0.198	0.164	0.172	1.217	0.091	0.113	0.611	8.385	18.118	10.320	5.756
P3	0.059	0.055	0.286	4.291	0.024	0.018	3.318	18.126	30.341	17.532	10.192
P4	0.862	6.392	22.034	33.653	0.199	0.254	24.804	17.161	82.063	61.907	46.310
P5	0.000	0.000	0.001	0.628	0.000	0.000	2.743	10.946	10.805	5.507	2.222
P6	0.020	0.002	0.105	1.181	0.009	0.009	1.051	15.895	39.693	21.381	10.844
P7	0.124	0.101	0.153	0.738	14.777	14.624	27.573	35.340	32.937	19.316	10.404
P8	0.000	0.001	0.001	3.178	0.135	0.184	8.817	44.628	54.132	43.122	30.894
P9	0.073	0.049	0.091	0.189	0.273	0.256	0.448	3.483	18.352	8.586	4.025
P10	0.094	0.077	0.105	0.804	0.077	0.086	1.093	9.358	14.948	6.113	3.042

Appendix F - GH Raw Data Post-Supplement

Group	TA1	TA2	TA3	TA4	TB1	TB2	TB3	TB4	TB5	TB6	TB7
	10 min pre	pre	post	10min post	10 min pre	pre	15 min	30 min	45 min	15 min post	30 min post
C1	0.109	0.081	0.095	1.078	0.076	0.102	0.165	6.346	12.745	8.512	9.997
C2	0.000	0.000	0.000	3.420	0.000	0.000	5.384	15.231	19.635	19.738	11.874
C3	0.173	0.229	1.397	8.452	0.219	0.233	8.042	26.306	31.529	16.443	7.602
C4	0.964	0.892	1.489	3.730	2.446	4.083	15.294	26.406	36.855	20.875	14.891
C5	0.045	0.032	0.054	4.291	0.609	3.575	22.753	30.140	37.319	19.291	9.879
C6	0.073	0.088	0.109	0.256	0.089	0.090	0.189	3.292	16.238	15.880	14.902
C7	0.065	0.077	0.069	0.224	0.141	0.078	1.019	12.746	25.892	11.300	5.510
C8	0.146	0.029	0.097	2.355	0.023	0.036	1.084	16.545	17.037	12.972	6.555
C9	0.072	0.054	0.091	3.304	0.107	0.167	2.875	22.419	29.496	14.557	6.087
C10	0.071	0.100	0.145	6.167	0.102	0.127	0.758	13.092	22.879	12.609	7.187
P1	0.046	0.054	0.065	0.303	0.057	0.041	0.987	11.754	18.319	5.753	2.095
P2	0.110	0.087	0.114	0.680	0.096	0.092	0.853	8.986	17.014	7.251	4.388
P3	0.018	0.012	2.807	10.818	0.016	0.016	2.246	21.251	33.750	12.784	5.627
P4	0.321	0.175	0.201	1.683	0.425	0.269	3.091	36.976	60.396	34.694	17.563
P5	0.000	0.003	0.004	0.749	0.002	0.000	1.566	7.339	10.660	6.022	2.402
P6	0.013	0.001	0.134	3.374	0.013	0.030	2.601	13.503	22.608	12.496	5.360
P7	0.268	0.139	0.240	2.358	0.130	0.120	1.178	11.203	18.442	7.866	4.837
P8	0.000	0.000	0.001	0.906	0.212	0.220	5.596	23.462	36.658	19.435	11.091
P9	0.073	0.063	0.085	0.847	0.287	0.233	0.996	11.188	42.309	17.579	8.661
P10	0.076	0.115	0.054	0.152	x	x	x	X	x	x	x

Appendix G Lactate Raw Data - Pre Supplement

Group	CA1	CA2	CA3	CA4	CB1	CB2	CB3	CB4	CB5	CB6	CB7
	10 min pre	pre	post	10min post	10 min pre	pre	15 min	30 min	45 min	15 min post	30 min post
C1	0.74	0.57	7.35	9.62	1.03	1.1	2.95	2.78	2.54	1.35	0.95
C2	1.07	0.73	10.45	12.82	1.47	1.17	5.05	3.74	3.49	1.78	1.42
C3	1.71	1.1	5.37	6.86	1.49	1.51	4.05	3.54	2.85	0.46	0.86
C4	0.72	0.79	6.38	6.55	0.3	0.4	1.87	2.26	2.45	0.27	0.62
C5	1.29	1.17	8.71	7.92	1.23	1.5	5	5.6	4.11	0.76	0.99
C6	0.8	0.8	6.1	10.02	0.94	0.99	2.56	4.02	2.27	0.41	0.71
C7	0.79	0.66	2.93	4.51	0.55	0.7	3.64	1.87	1.17	0.19	0.53
C8	1.1	1.12	9.14	15.73	0.71	0.66	3.97	3.94	5.02	1.8	1.35
C9	0.83	0.81	7.48	6.61	0.63	0.5	3.22	3.44	3.17	0.36	0.84
C10	1.66	1.12	8	9.45	1.05	1.02	6.31	7.34	9.02	3.68	2.37
P1	x	x	x	x	1.51	1.38	8.62	10.82	7.75	4.42	3.04
P2	1	0.78	6.53	10.22	1.05	0.94	3.39	1.14	2.52	0.71	0.82
P3	1.32	0.85	7.95	13.28	1.24	1.11	4.54	5.41	5.27	0.75	1.45
P4	1.21	1.26	5.96	10.34	1.22	1.07	6.74	7.98	7.6	1.33	2.55
P5	0.86	0.63	5.47	10.56	1.16	0.91	6.44	6.05	5.72	0.98	1.7
P6	1.43	1.13	10.13	13.01	1.18	1.01	5.07	5.55	5.91	2.73	1.64
P7	1.46	0.9	11.02	11.99	0.96	0.66	2.9	7.94	7.22	1.36	2.57
P8	1.1	1.16	7.58	7.27	1.48	1.54	8.18	6.71	5.86	2.98	1.52
P9	1.49	1.07	9.66	12.41	1.27	1.22	5.93	6.45	6.1	2.5	1.88
P10	1.495	1.07	6.86	5.63	x	x	x	x	x	x	x

Appendix H - Lactate Raw Data Post-Supplement

Group	TA1	TA2	TA3	TA4	TB1	TB2	TB3	TB4	TB5	TB6	TB7
	10 min pre	pre	post	10min post	10 min pre	pre	15 min	30 min	45 min	15 min post	30 min post
C1	0.74	0.63	8.64	6.86	0.8	0.73	2.36	3.41	2.84	1.34	1.16
C2	1.26	1.09	11.31	10.9	1.46	1.46	6.56	6.58	8.37	4.35	3.02
C3	1.17	0.77	9.85	7.07	1.87	1.74	6.15	5.3	4.39	0.6	1.32
C4	0.56	0.6	5.81	6.84	0.68	0.54	2.93	3.5	4.46	0.67	0.97
C5	1	1.11	5.84	9.82	0.96	0.74	3.86	4.05	3.76	0.53	1.12
C6	0.9	1.13	8.31	12.44	1.17	0.98	2.75	2.78	2.33	0.23	1
C7	1.21	1.07	7.95	7.41	1.52	1.01	5.35	4.26	2.45	0.34	1.05
C8	1.16	0.81	7.3	13.55	1.12	0.94	4.9	5.12	4.93	1.78	1.21
C9	0.79	0.56	8.37	11.11	0.86	0.77	2.71	2.84	2.24	0.3	0.78
C10	1.02	1.16	10.42	11.56	1.12	1.19	6.77	6.46	6.12	3.25	2.46
P1	x	x	x	x	1.23	1.07	6.02	5.21	5.57	2.2	1.35
P2	0.92	0.96	6.29	8.79	1.01	0.87	3.13	3.03	1.98	0.23	0.8
P3	1.41	1.03	12.35	15.13	1.36	1.19	5.57	7.45	5.72	0.81	1.57
P4	1.4	1.17	7.58	11.08	1.28	1.23	6.4	6.83	7.13	1.1	2.65
P5	0.63	0.57	6.25	8.35	0.72	0.75	4.24	4.26	5.38	0.7	0.98
P6	0.88	0.44	9.84	14.9	1.24	0.77	3.9	4.12	2.81	1.19	0.54
P7	1.13	0.85	7.66	14.37	1.04	1.01	5.66	7.06	5.8	0.87	1.65
P8	1.25	1.33	3.48	5.93	1.2	0.98	5.66	5.11	4.73	1.68	1.68
P9	1.34	0.96	11.18	12.17	1.44	1.14	6.65	6.76	5.85	1.82	1.35
P10	1.54	1.13	6.94	6.44	x	x	x	x	x	x	x

Appendix I Glucose Raw Data - Pre Supplement

Group	CA1	CA2	CA3	CA4	CB1	CB2	CB3	CB4	CB5	CB6	CB7
	10 min pre	pre	post	10min post	10 min pre	pre	15 min	30 min	45 min	15 min post	30 min post
C1	4.76	3.96	5.41	5.87	3.05	2.81	3.39	3.08	3.29	3.95	4.25
C2	2.90	3.66	4.39	5.17	4.62	4.26	3.59	3.17	3.45	4.85	3.82
C3	4.55	3.81	2.73	3.90	4.52	4.42	2.93	3.69	3.7	3.87	3.71
C4	4.24	3.84	3.61	5.78	4.5	4.8	5.85	3.39	3.19	5.58	4.13
C5	3.14	3.84	3.73	3.60	3.32	3.87	2.57	2.85	3.76	4.85	3.87
C6	2.68	2.59	3.12	3.62	1.74	1.95	1.81	3.35	2.72	3.52	2.69
C7	2.93	2.37	2.99	3.30	4.93	4.34	3.15	3.44	4.19	5.34	9.21
C8	3.25	3.27	3.05	4.76	3.05	3.02	3.13	3.38	3.95	3.87	5.07
C9	2.40	2.68	2.45	3.07	3.06	2.69	2.34	3.22	3.33	4.10	4.26
C10	3.18	3.01	3.04	3.16	4.42	4.31	2.24	2.68	3.59	3.86	3.72
P1	x	x	x	x	3.05	2.33	1.97	4.19	3.84	5.29	4.75
P2	3.13	2.95	1.95	3.25	3.19	2.59	3.58	4.93	4.66	4.41	4.09
P3	3.94	3.33	2.39	3.45	2.8	2.7	3.48	4.15	3.94	4.27	4.52
P4	2.95	3.37	3.78	4.48	3.01	3.02	3.16	4.01	4.48	3.96	4.19
P5	2.57	2.14	2.46	3.66	3.82	3.67	3.26	3.73	4.17	4.85	4.81
P6	3.69	3.83	3.02	3.33	3.45	3.52	3.15	3.61	3.21	4.11	4.23
P7	3.92	2.54	3.25	4.04	3.07	2.44	2.29	3.68	4.14	4.67	4.9
P8	1.71	3.17	3.09	3.18	2.96	2.98	3.8	4.16	3.92	4.26	3.99
P9	3.16	2.63	2.49	2.63	2.49	3.97	3.67	3.06	3.05	2.81	3.39
P10	4.36	4.62	3.87	4.76	x	x	x	x	x	x	x

Appendix J - Glucose Raw Data - Post-Supplement

Group	TA1	TA2	TA3	TA4	TB1	TB2	TB3	TB4	TB5	TB6	TB7
	10 min pre	pre	post	10min post	10 min pre	pre	15 min	30 min	45 min	15 min post	30 min post
C1	2.23	2.10	2.50	2.81	3.22	2.33	2.41	2.63	3.16	3.26	3.32
C2	2.96	3.92	3.24	4.20	2.50	2.85	4.09	4.15	4.76	4.48	5.17
C3	3.43	4.62	2.83	2.89	4.48	4.38	3.09	4	4.12	5.53	4.32
C4	3.96	3.39	4.83	3.89	4.78	4.12	3.37	3.39	3.58	3.82	4.48
C5	3.06	3.42	2.27	2.63	4.16	4.22	2.41	3.59	3.81	3.77	3.82
C6	2.38	2.69	2.59	3.08	3.01	2.96	3.05	3.18	3.18	3.69	3.83
C7	3.64	3.83	3.31	3.47	3.46	3.75	3.04	3.39	4.14	5.02	4.83
C8	2.89	2.30	3.12	4.65	2.1	2.41	3.16	4.14	3.52	4.94	3.99
C9	3.29	3.32	2.75	3.23	2.82	2.34	3.13	3.52	3.46	4.83	3.97
C10	2.85	2.90	3.25	3.39	2.83	3.11	2.87	3.64	4.69	4.26	4.1
P1	x	x	x	x	2.35	2.49	2.89	3.79	3.91	5.54	4.58
P2	3.76	3.59	3.06	4.24	4.01	3.92	3.33	4.35	4.83	5.35	4.91
P3	3.65	3.32	3.46	3.78	2.69	3.01	2.84	3.29	3.78	5.21	5.45
P4	2.92	2.57	2.47	3.68	3.19	3.52	3.37	4.55	4.56	4.98	5.78
P5	2.99	2.92	2.54	3.09	3.93	3.39	3.02	3.66	4.04	4.43	4.24
P6	2.11	4.23	2.11	1.22	2.64	3.74	3.02	2.7	2.98	3.49	3.39
P7	3.32	2.43	2.63	4.21	3.25	3.59	3.04	3.36	3.49	4.02	4
P8	2.49	2.68	1.97	2.75	2.58	2.54	3.4	3.96	3.55	4	4.35
P9	4.16	4.02	4.25	3.30	3.29	4.32	3.51	3.1	2.61	3.04	4.04
P10	3.59	3.06	2.98	3.59	x	x	x	x	x	x	x

Appendix K - MAOD Raw Data

Creatine Group				Placebo Group			
Subject	Familiarization	Pre-Supplement	Post-Supplement	Subject	Familiarization	Pre-Supplement	Post-Supplement
C1	3.95	4.02	4.09	P1	3.56	3.88	4.01
C2	3.24	3.25	5.07	P2	1.75	2.79	2.392
C3	2.72	2.89	3.42	P3	5.98	7.30	7.156
C4	4.11	4.19	4.65	P4	3.30	3.50	3.523
C5	5.40	5.46	5.55	P5	3.67	3.61	3.58
C6	4.80	5.16	5.32	P6	2.98	3.01	3.534
C7	1.40	1.50	2.14	P7	1.89	2.00	3.012
C8	2.06	2.84	3.64	P8	1.69	1.83	2.31
C9	3.07	3.38	4.26	P9	2.72	3.36	4.059
C10	3.02	3.10	4.42	P10	2.72	2.92	2.941