THE EFFECT OF ORAL CREATINE SUPPLEMENTATION ON ONE HOUR CYCLING PERFORMANCE AND METABOLISM

BRETT BELLINGER

SUPERVISOR: DR KATHRYN MYBURGH

Submitted to the Faculty of Medicine at the University of Cape Town

in fulfillment of the requirements of the

Masters degree in Medicine (Exercise Science)

December 1995

TABLE OF CONTENTS

TABLES

FIGURES

REFERENCES

APPENDICES

ACKNOWLEDGEMENTS

First, and foremost I would like to acknowledge Toni Bold for the great times and friendship in spite of numerous research 'hazards'. Secondly, thanks to Alexa for the best six months of the entire thesis. In addition, thanks must go to Kathy Myburgh who guided me through my whole Masters and red-penned many a draft copy.

Thanks and heartfelt appreciation to Gary Wilson and Judy Belonje for advice in setting up the HPLC methods and performing the lactate assays (as well as chats over numerous cups of coffee).

Thanks to all the Doctors: Zig, Hunter, Tim, Mike and Martin for all the biopsies especially at those 5am slots ! Thanks to the FRD for the bursary and to Arcoen c.c. for the sponsorship to do the research, and to all those others in the Dept. who aided me with advice, humour and inspiration.

Finally, thanks to my Mother who has made all my studying possible and without whose guidance this would not have been achieved. This document is dedicated to her.

DECLARATION

I, BRETT BELLINGER, declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part thereof has been, is being, or is to be submitted for any other degree at this or any other University.

I empower the University of Cape Town to reproduce for the purpose of research the whole, or any portion of the contents in any manner whatsoever.

Signed by candidate

BRETT BELLINGER

CAPE TOWN

ABSTRACT

The effect of oral creatine (Cr) supplementation with 20 g/ day for 7 days on one-hour cycling performance and metabolism was investigated in a double-blind placebo controlled study. Twenty endurance-trained male cyclists volunteered for the study that was approved by the University of Cape Town ethics committee. The subjects' peak sustained power output was measured and they underwent a familiarization one-hour cycle on a cycle ergometer. Five days later subjects had a muscle biopsy and an in-dwelling cannula was inserted into a forearm vein before performing a maximal one-hour cycle (T1) during which blood samples were taken at regular intervals. Following the cycle, the subjects each received either Cr or placebo tablets to be ingested four times per day for the following week. After the loading week the subjects again reported to the laboratory, had a muscle biopsy and underwent and the same test routine (T2) with blood sampling as the previous week. Resting muscle total Cr measured by HPLC increased significantly ($p<0.001$) in the Cr group from 123.0 ± 3.8 mmol/ kg dry wt to 159.8 ± 7.9 mmol/ kg dry wt, but was unchanged in the placebo group. The extent of Cr loading was not related to baseline Cr levels ($r=0.46$, NS). No differences were noted in the resting levels of adenine nucleotides in either group. Analyses of the plasma samples indicated no change in plasma lactate concentration, but a significant lowering of indicators of adenine nucleotide degradation including concentrations of plasma ammonia ($p<0.05$) and hypoxanthine ($p<0.01$) in the Cr group from T1 to T2. Plasma urate concentration was significantly lowered ($p<0.01$) from T1 to T2 but there were no differences between groups. Cr supplementation did not significantly improve performance in the one-hour trial (Cr group : 39.1 ± 0.9 km vs 39.8 ± 0.8 km and placebo group : 39.3 ± 0.8 km vs 39.2 ± 1.1 km). We hypothesised that Cr supplementation affects the purine nucleotide cycle through improved maintenance of low intracellular ADP during exercise as a result of enhanced efficiency of the Cr-PCr shuttle. Cr supplementation had no effect on lactate and carbohydrate metabolism and did not improve performance significantly during a one-hour cycle trial.

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: RATIONALE AND AIMS

CHAPTER 1: RATIONALE AND AIMS

Supplementing human diets with creatine (Cr) is not a new concept. Studies by Crim et al., (1976) have examined the size of the body Cr pool and its effect on Cr turnover through supplementation procedures combined with radioactive tracer labeling techniques. In addition, the effect of oral Cr supplementation has been studied in patients with a Cr deficiency such as those with gyrate atrophy of the choroid and retina (Sipila et al., 1981). However, it is only in recent years that several groups have examined the effect of Cr supplementation on muscle Cr content and athletic performance.

The role that Cr in its phosphorylated form, phosphocreatine (PCr), plays in the body is primarily one of an 'energy reservoir' to regenerate adenosine triphosphate (ATP) which has been hydrolysed to adenosine diphosphate (ADP). It is not surprising that most of the body's PCr is found in muscle, since the rate of ATP turnover in this tissue is greatly increased by exercise. The rapid regeneration of ATP in muscle by PCr hydrolysis during exercise prevents reductions in the ATP:ADP ratio that would otherwise decrease the transfer of free energy to the myosin heads of the thick myofilaments in the myofibrils. ATP regeneration by nett PCr hydrolysis is particularly important during intense, maximal activity when ATP turnover is at its highest.

Therefore the first studies examined the effects of additional Cr ingestion on exercise performance in maximal exercise or on several intermittent bouts of maximal or near maximal exercise. Some of these studies showed performance enhancement (Balsom et al., 1993a; Birch et al., 1994; Earnest et al., 1994 (Abstract); Greenhaff et al., 1993a; Lemon et al., 1995 (Abstract); Soderlund et al., 1994 (Abstract); Viru and Nurmeviki, 1993) and others failed to show any improvements (Almada et al., 1995 (Abstract); Balsom et al., 1993b; Burke et al., 1995 (Abstract); Cooke et al., 1995; Grindstaff et al., 1995 (Abstract); Odland et al., 1994 (Abstract); Redondo et al., 1995 (Abstract); Stroud et al., 1994). It is unknown whether these negative results were due to poor uptake of the ingested creatine, since individual responses to creatine loading are variable (Harris et al., 1992; Lemon et al., 1995 (Abstract)). Teleologically it would make more sense for a sprinter who utilises the phosphagen energy system to a much greater extent than an endurance athlete, to have higher muscle TCr. Spriet (1995) summarised data showing that PCr content of Type II fibres to be 5 - 15 % higher than in Type I fibres. Therefore, if endurance athletes have more Type I fibres

than sprinters, their baseline PCr content may also be different. Since Harris et al., (1992) have shown that low baseline levels enhance muscle Cr uptake during supplementation, endurance athletes may have a greater retention of the Cr supplement than previously shown in more generally active subjects. This however has not been determined since few studies have been performed on endurance athletes and Cr supplementation, and none of these have performed muscle biopsies for determination of muscle Cr concentration.

In some of these studies, the authors also investigated the effects of Cr supplementation on metabolic changes associated with fatigue during exercise (Balsom et al., 1993a; Balsom et al., 1993b; Birch et al., 1994; Greenhaff et al., 1993a; Stroud et al., 1994; Soderlund et al., 1994 (Abstract)). Results indicated that plasma ammonia (NH_3) levels were lowered (Birch et al., 1994; Greenhaff et al., 1993a) and plasma hypoxanthine (Hx) levels were lowered (Balsom et al., 1993a) or unchanged (Balsom et al., 1993b) as a result of Cr loading. However, plasma lactate was lowered in two studies (Balsom et al., 1993a; Soderlund et al., 1994 (Abstract)), but not in others (Balsom et al., 1993b; Birch et al., 1994; Greenhaff et al., 1993a; Stroud et al., 1994). Evidently Cr supplementation is in some way altering metabolism during exercise and the paradigm arising from the initial studies is that the main effect of Cr supplementation is to reduce adenine nucleotide degradation during high intensity, maximal or near maximal exercise.

The fact that two studies have shown decreases in plasma lactate with Cr loading implies that increased muscle PCr levels may influence carbohydrate (CHO) metabolism as well. This may be related to the duration of the exercise. In addition, Cr may play a role in the regulation of the rate of mitochondrial ATP production (Jacobus, 1985; Korge and Campbell, 1995; Radda et al., 1995). If CHO metabolism and mitochondrial function are altered through Cr supplementation, it is possible that exercise performance at submaximal intensities may also be influenced. Evidence in support of an effect of Cr concentration on mitochondria is given by the enhanced rate of PCr rephosphorylation during rest after Cr supplementation (Greenhaff et al., 1993b).

Several studies not examining the effects of Cr supplementation have shown appreciable ammonia (Bouckaert and Pannier, 1995; Brouns et al., 1990) and lactate accumulation (Palmer et al.,unpublished data) during endurance exercise lasting approximately one hour. There is also substantial evidence that high ammonia accumulation is often accompanied by high lactate

accumulation (Bouckaert and Pannier, 1995; Urhausen and Kindermann, 1992). It is thought that as Cr has been shown to have an effect on these measures during maximal exercise, there may be a similar effect in high intensity submaximal exercise. Thus, should PCr rephosphorylation also be enhanced *during submaximal* exercise, a role for Cr supplementation for endurance athletes may be warranted in terms of alterations in adenine nucleotide and carbohydrate metabolism possibly translating into improved performance. This is thought to be via the increase in PCr lowering the accumulation of adenosine diphosphate (ADP), adenosine monophosphate (AMP), ammonia and inosine monophosphate(IMP). These compounds are all participants in adenine nucleotide metabolism and are also allosteric activators of glycolytic activity. Therefore a lessening in the accumulation of these products will affect both adenine nucleotide and carbohydrate metabolism, indicated in lower levels of plasma ammonia and blood lactate. Thus, it needs to be investigated whether the effects seen in adenine nucleotide and carbohydrate metabolism during maximal exercise are also evident in high-intensity submaximal exercise where similar systems of energy provision are affected but to a lesser degree.

It should be noted, however, that the use of PCr as a fuel substrate is not limiting during endurance exercise since the rate of ATP demand is not high enough. Thus, in endurance exercise, the role of Cr as an ergogenic aid either would be as a 'high energy' phosphate carrier or by improving the efficiency of 'high energy' transfer from ATP to Cr at the mitochondrial membrane and from PCr to ADP at the myofibrils. This may alter metabolism sufficiently to decrease some factors related to fatigue which are not related to substrate availability, for example, ADP and Pi concentrations. Perception of relative fatigue during sustained high intensity exercise (as opposed to perception of fatigue sufficient to halt exercise completely) is likely to influence the chosen gear ratios and pace, both of which may influence the specific exercise task utilised in this study.

Accordingly, the aims of this study were to examine the effect of oral creatine supplementation on i) muscle creatine content in well-trained endurance athletes, ii) high-intensity endurance exercise performance during a cycling distance trial lasting one-hour and iii) adenine nucleotide and carbohydrate metabolism during the cycling trial and recovery.

CHAPTER 2: INTRODUCTION

CHAPTER 2: INTRODUCTION

2.1 THE ENERGY SYSTEMS UTILISED DURING EXERCISE

Muscular contraction is an energy requiring process which involves the interaction between the thick and thin filaments (the contractile proteins) that constitute a muscle fibre (McArdle et al., 1991). This involves the hydrolysis of ATP to ADP with the subsequent release of phosphate (Pi), ADP and free energy. The latter is utilised for the conformational changes in myosin that result in force production (Cooke, 1986). In order to provide energy for muscular activity various metabolic pathways that resynthesise ATP from ADP are activated. It is important to maintain an optimal ATP concentration as well as a high ATP:ADP ratio in order for contraction to continue. These metabolic pathways are activated to varying degrees depending on both the intensity and duration of the exercise bout (Astrand and Rodahl, 1970; Hawley and Hopkins, 1995). These pathways or energy systems include the phosphagen (ATP and PCr hydrolysis) system, oxygen-(O_2) independent glycolysis, O_2-dependent CHO oxidation, O_2-dependent lipid oxidation and to some extent protein oxidation (Hawley and Hopkins, 1995). In addition, another system called the purine nucleotide cycle (PNC) may be activated (Tullson and Terjung, 1991). The major function of this system is not to produce ATP, but rather to remove excess ADP in order to maintain an optimal ATP:ADP ratio.

2.1.1 THE PHOSPHAGEN SYSTEM

This system stores energy in the form of 'high energy' phosphate bonds and this energy can be liberated by hydrolysis conformational change and product release. Myosin ATPase hydrolyses ATP to ADP and Pi. The free energy released at pH 7 is high, -7.3 kcal/ mol (Stryer, 1995). A 'high energy' phosphate bond also exists between Cr and Pi when it is present as the phosphagen PCr. PCr hydrolysis itself releases -10.3 kcal/ mol of free energy (Stryer, 1995). PCr is used for immediate resynthesis of ADP to ATP through a 'high-energy' phosphate transfer reaction catalysed by a myofibrillar creatine kinase (CK). Approximately 60 to 66.8% of the total Cr (TCr) exists as PCr in non-working muscles (Harris et al., 1974; Harris et al., 1992).

Energy demand of muscle contraction results in decreased levels of ATP and increased levels of ADP and the equilibrium of the CK reaction then favours ATP formation (Sweeney, 1994). The CK reaction operates at high levels of efficiency and does not utilise oxygen. PCr is therefore a

major substrate for rapid resynthesis of ATP in the muscle, particularly during very high intensity, short duration exercise (McArdle et al., 1991). This is because of the proximity of the CK enzymes to the contractile proteins and the ease with which the activity of CK can be increased by small decreases in ATP concentrations (Sweeney, 1994). This function of PCr as an immediate buffer to energy use is limited by its fairly low concentration.

Regeneration of the PCr supply is an oxidative process occuring in the mitochondria where a different isoform of CK exists than that found at the myofibrils. Thus, CK is found in close association with both the contractile proteins of the myofibrils and the mitochondria. Recent research has shown that the 'shuttle' has more functions than simply as a buffer to energy use and a transport network (Sweeney, 1994; Walliman et al., 1992). It has also been found to be vital in: preventing rises in ADP which among other things is a precursor to adenine nucleotide loss, preventing increases in hydrogen ion concentrations, indirectly regulating glycogenolysis and glycolysis through the release of inorganic phosphate (Pi), regulating the ATP:ADP ratio and as a regulator of mitochondrial respiration. For these reasons Walliman et al., (1992) suggest that the 'Cr-PCr shuttle' be renamed the 'PCr circuit'as this term implies more functions than simply transport.

Several recently published studies (Jacobus, 1985; Thompson et al., 1995; Radda et al., 1995; Wilson, 1994; Meyer and Foley, 1994; Walliman et al., 1992) have indicated that these functions are the subject of much debate especially in the light of research (Meyer et al., 1986; Wyss and Walliman, 1994) on beta-guanadinopropionate (a Cr analogue) fed rats, which has indicated that simple diffusion of ATP is a sufficient energy translocation process under most conditions. This probably indicates that the 'shuttle' or 'PCr circuit' is not essential for steady state energy production but does seem to be important in regulating glycolytic process through the release of phosphate by PCr hydrolysis. The 'shuttle' does seem to provide a far more efficient method of energy translocation than by the simple diffusion of ATP. Thus, for the purposes of this discussion, the terms 'Cr-PCr shuttle' and 'PCr circuit' will be used interchangeably and both should be recognised as having all functions discussed above.

The absolute intramuscular contents of Cr and PCr are important metabolic regulators of the other energy provision pathways. Decreased levels of PCr and increased levels of Cr are two of the

important intracellular signals that the ATP demand of the exercise is such that more sustainable processes of energy provision are required. Decreased levels of PCr and increased levels of Cr aid in the regulation of substrate cycling that occurs during the acceleration of O_2-independent CHO metabolism by enhancing the rate of the phosphofructokinase (PFK) reaction as well as glycogen phosphorylase activity (as reviewed by Spriet, 1995). This occurs both directly where Cr and PCr regulate PFK and glycogen phosphorylase, and indirectly where the Pi liberated from PCr hydrolysis also causes activation of these enzymes. Thus, Cr and PCr play a role in regulating glycolytic and glycogenolytic activity. Alternatively Cr also aids the aerobic systems through its action as a rapid transportation system of 'high-energy' phosphate from the mitochondria (the site of aerobic ATP synthesis) to the myofibrils (the site of ATP use). This reaction is mediated by mitochondrially bound CK and ensures that ATP does not accumulate intra-mitochondrially and that ADP is always readily available for subsequent oxidative rephosphorylation and is therefore indirectly a participant in the regulation of mitochondrial respiration (as reviewed by Hargreaves, 1995). Thus, Cr and PCr can be seen important participants in both O_2-independent and O_2-dependent metabolism.

The relative contribution of this energy yielding process differs not only according to the intensity and duration of the exercise, but also the length of rest periods between exercise bouts (Gaitanos et al., 1993; Spencer et al., 1991; Hawley and Hopkins, 1995). The contribution of PCr is particularly dependent on the level of its store in the contracting muscle (Harris et al., 1992). The free energy released from PCr hydrolysis is sufficient to last for only approximately 6 seconds or less of maximal high intensity exercise (Gaitanos et al., 1993). During intermittent exercise, should the rest period not be long enough for full PCr resynthesis, each successive bout will begin with lower PCr and therefore less of a reliance on this energy store. When most PCr has been hydrolysed, a greater demand is placed on O_2-independent glycogenolysis and glycolysis (Urhausen and Kindermann, 1992).

In summary, if Cr is only rephosphorylated in resting muscle, then there would be no role for the 'Cr-PCr shuttle' or 'PCr circuit' during exercise and the contribution of PCr to ATP maintenance could be calculated directly from the decline in PCr from rest to exhaustion. If this were the case, then all other ATP would diffuse from glycolytic sites or from the mitochondria to the myofibrils. However, it seems likely that there should be a turnover of PCr which is made possible by the

'shuttle' and therefore, the level of PCr seen during more prolonged exercise is a steady state concentration which is influenced by the rates in the following sequence: rate of PCr utilised for ADP rephosphorylation near the myofibrils, rate of Cr diffusion from the myofibrils back to the mitochondria, rate Cr can be rephosphorylated at the mitochondrial membrane and the rate of diffusion of PCr back to myofibrils.

2.1.2 THE O_2-INDEPENDENT GLYCOLYTIC SYSTEM

While the reactions of the phosphagen system can be accelerated rapidly, the glycolytic pathway takes several seconds to be activated. Its activation is dependent largely on the demand of ATP being so high that large amounts of ADP accumulate and are subsequently deaminated to AMP, this occurs simultaneously with decreases in PCr and the resulting increases in Cr and Pi. The accumulation of ADP, AMP, IMP and Pi in particular cause the enzyme, glycogen phosphorylase b to convert to its more active form glycogen phosphorylase a, also increasing its activity and thereby stimulating glycogenolysis (as reviewed by Spriet, 1995). The products of ATP utilisation (excluding IMP) also cause the allosteric activation of PFK, thereby stimulating glycolysis by increasing the flux through the PFK reaction (as reviewed by Spriet, 1995).

This pathway produces ATP from the breakdown of muscle glycogen and plasma glucose without oxygen consumption and is therefore the pathway that is activated to a large extent when exercise is so intense that neither the phosphagen system nor the oxidative processes can keep up with the ATP demand. Alternatively, it is the pathway activated early in exercise before cardiac output and muscle blood flow increase. Concomitant with the production of ATP is the formation of lactate and the release of hydrogen ions. Thus, the level of lactate in the blood can be seen to be an indicator of the extent of O_2-independent glycolytic activity. Hydrogen ion formation causes a drop in intracellular pH and this has a direct effect on the acto-myosin cross-bridges by decreasing force production (Cooke et al., 1988) and work output over time (de Haan et al., 1989). Hydrogen ions are allosteric inhibitors of phosphorylase and PFK, however, their effect seems to be largely negated by the strength of the allosteric activators of these enzymes (as reviewed by Spriet, 1995). It is therefore hypothesised that an increased availability of PCr may decrease hydrogen ion formation through less activation of the glycolytic processes and therefore help with the maintenance of force output in the working muscle. The phosphate liberated by PCr

breakdown also increases the buffering capacity of the working muscle which should further help to reduce intracellular hydrogen ion accumulation (Harris et al., 1976).

The energy produced as a result of the activation of O_2-independent glycolysis is sufficient to last for approximately 1 minute of intense exercise before Pi and hydrogen ion accumulation cause fatigue. (Hawley and Hopkins, 1995). For more sustained activity, therefore, Pi accumulation from nett PCr breakdown has to be limited and hydrogen ions from glycolytic ATP turnover have to be transported into the mitochondria together with pyruvate. In addition, ATP must be produced by mitochondrial oxidative phosphorylation which is fueled by both CHO and fat. This will occur at lower exercise intensities than those which rely on non-oxidative pathways.

2.1.3 THE AEROBIC GLYCOLYTIC AND LIPOLYTIC SYSTEMS

The duration and intensity of the activity and the substrate availability will determine the predominant fuel that is used during sub-maximal exercise (Hawley and Hopkins, 1995). The O_2-dependent glycolytic system will provide most of the energy for short-term endurance exercise (30 minutes - 2 hours) while the O_2-dependent lipolytic system is increasingly used for more prolonged activities (2 hours and longer) due to either further decreases in exercise intensity, decreasing CHO availability or metabolic and hormonal activation of lipolysis (Hawley and Hopkins, 1995). Thus, unless CHO is ingested, the lower the exercise intensity or the longer the duration, the greater the dependence on lipid relative to carbohydrate (Edwards et al., 1934). The aerobic systems are also termed oxidative or O_2-requiring processes.

Therefore, as stated previously, the major aim of these four systems is to provide ATP for contraction through rephosphorylation of ADP and through this maintain the ATP:ADP ratio at the myofibrils at optimal levels. However, another process may be activated in order to maintain an optimal ATP:ADP ratio for contraction. This process removes excess ADP that accumulates, as a result of an inability to rephosphorylate ADP fast enough, by degrading it.

2.1.4 THE PURINE NUCLEOTIDE CYCLE

Failure of the energy systems to rephosphorylate ADP to ATP displaces the myokinase equilibrium towards AMP formation: ADP + ADP $\leftrightarrow$ATP + AMP. The AMP can be deaminated to inosine monophosphate (IMP) by AMP deaminase with the release of ammonia (NH_3). It

should be noted that ADP, AMP, IMP are regulators of glycogen phosphorylase b and phosphorylase a and that ADP,AMP and the ammonium ion, NH_4^+ are regulators of PFK (Hargreaves, 1995; Spriet, 1995;Tullson and Terjung, 1991) and through these the PNC is involved in CHO metabolism.

In the activation of the purine nucleotide cycle (PNC), the rises in ADP, Pi and hydrogen ions resulting from high energy demand and the use of CHO cause the allosteric activation of AMP deaminase (Lowenstein, 1990). The NH_3 produced from the AMP deaminase reaction subsequently appears in the blood and has been used as an indicator of the degree of adenine nucleotide degradation and AMP deaminase activity (Banister and Cameron, 1990; Broberg and Sahlin, 1989; Graham et al., 1990; Sewell and Harris, 1992). The extent of NH_3 release is dependent on the exercise intensity, duration and also on the exercise mode (Bouckaert and Pannier, 1995). Large quantities appear in the blood following highly intense or very long periods of exercise (Urhausen and Kindermann, 1992). Resting levels are reported to be from 20 to 25 µmol/ l in venous blood (Banister and Cameron, 1990). Values of up to 250 µmol/ l have been reported after highly intense cycling lasting up to 5 hours (Brouns et al., 1990). Katz et al., (1986) have reported resting levels of 95 µmol/ l increasing up to approximately 220 µmol/ l following a 10 minute warm-up at 50 % VO_{2max} and a subsequent maximal cycle at 100% of VO_{2max}. However in exercise of long duration at least some of the plasma NH_3 may be due to amino acid catabolism (Banister and Cameron, 1990).

One of the consequences of an increase in plasma NH_3 is that NH_3 crosses the blood-brain barrier by diffusion (Banister and Cameron, 1990). In clinical conditions of elevated plasma NH_3, symptoms of central and peripheral fatigue become evident (Banister et al., 1985; Wagenmakers, 1992). Thus far however, scientific evidence for exercise-induced elevated plasma NH_3 resulting in the development of central fatigue is inconclusive.. Research seems to implicate elevated plasma NH_3 with the development of peripheral fatigue in as much as elevated muscle NH_3 stimulates glycolytic activity and therefore depletes limited CHO stores (Wagenmakers, 1992).

Muscle fibre type and recruitment also influence NH_3 production. The fibres which have the highest AMP deaminase activity (Type IIB) also produce the most NH_3. The greatest NH_3 production therefore occurs during exercise which activates Type IIB fibres, such as during high-

intensity near-maximal or maximal activity in human subjects (Broberg and Sahlin, 1989; Dudley and Terjung, 1985a; Dudley and Terjung, 1985b; Meyer and Terjung, 1980; Tullson et al, 1990; Tullson and Terjung, 1990) and intense stimulation of fast twitch muscle in animal models (Meyer et al., 1980).

The IMP produced from AMP deamination may be broken down subsequently to hypoxanthine (Hx), xanthine (X) and urate (Ur) or alternatively reaminated in a two-step process to AMP (Lowenstein, 1990) (see Figure 2). Reamination of IMP is an energy requiring process, which utilises GTP, but reduces the potential loss of adenine nucleotides from the cell and to some extent restores muscle ATP levels (Broberg and Sahlin, 1989; Tullson and Terjung, 1990). The reamination of IMP seems to operate mainly during recovery following intense short duration exercise (Meyer and Terjung, 1980) or alternatively, the deamination and reamination pathways may act in concert, but not simultaneously, as a cycle during more moderate exercise (Lowenstein, 1990; Tullson and Terjung, 1991). This is because adenylosuccinate synthetase is activated with relatively low IMP accumulation, but is inhibited by high levels of IMP (Tullson and Terjung, 1991).

Hx, X and Ur can diffuse from muscle to blood and are therefore also used as blood-based markers of adenine nucleotide degradation (Bangsbo et al., 1992; Sewell and Harris, 1992; Stathis et al., 1994; Sutton et al., 1980). Despite its diffusion capacity, muscle Hx represents the final point at which adenine nucleotides can be recycled back to IMP via a pathway called the Purine Salvage Pathway and subsequently via the reaminating limb from IMP to AMP and then to ATP (Tullson and Terjung, 1991). The breakdown reactions to X and Ur are one-way reactions ultimately resulting in a nett loss of adenine nucleotides from the cell. These lost adenine nucleotides are only slowly and energetically expensively replaced through de novo synthesis (Tullson et al., 1988; Tullson and Terjung, 1990; Tullson and Terjung, 1991). This entire system of deaminating and reaminating cycles is termed adenine nucleotide metabolism (ANM) or the PNC.

2.2 CONTRIBUTIONS OF THESE SYSTEMS DURING ONE-HOUR EXERCISE

Depending on the duration and intensity of the exercise bout, some or all of the energy systems may be in operation at one time. For example, a 3 hour endurance event at approximately 70% VO_{2max} the body will tend to rely largely but not exclusively on O_2-dependent oxidation of CHO. However, phosphagen and O_2-independent glycolysis may contribute significantly to ATP resynthesis early on in exercise or intermittently with a change in pace and aerobic lipolysis may become increasingly active as the exercise tends to 3 hours if the subjects become CHO depleted. (Hawley and Hopkins, 1995)

During shorter events lasting approximately one-hour, the exercise intensity is such that the energy demand is high for the entire hour (typically 89 % of maximum heart-rate for a 40 km time trial - Palmer et al., 1995). Under these circumstances, it is likely that greater activation of the phosphagen system will occur at the onset of the exercise bout as well as intermittently during the course of the hour with changes in pace. However, O_2-independent and O_2-dependent CHO degradation will be the two main energy sources. It is not known to what extent adenine nucleotide degradation will occur at this pace (Katz et al., 1986). Thus, one of the contributors to metabolism in one-hour exercise is the phosphagen system and its associated functions which facilitate the faster transport of 'high-energy' phosphagens from the mitochondria to the myofibrils than would be possible by simple diffusion of ATP alone (see 2.1.1). Thus, were it not for the efficient operation and integrated action of the energy systems and adenine nucleotide control, it would be difficult for exercise to continue at a very high intensity for an hour (Astrand and Rodahl., 1970, Hawley and Hopkins, 1995).

2.3 THE EFFECT OF CREATINE SUPPLEMENTATION ON PERFORMANCE AND METABOLISM

2.3.1 GENERAL

A 70 kg man has on average 120 g of total Cr (TCr, the sum of Cr and PCr) stored in his body (Crim et al., 1976). This pool of Cr is replenished at a rate of approximately 2 g per day to replace the Cr that is lost through the daily non-enzymatic breakdown of Cr to creatinine. Cr is

replaced either through biosynthesis or an uptake of dietary Cr (Walker, 1979). Cr is synthesised from the amino acids arginine, glycine and methionine (Crim et al., 1976; Walker, 1979) and this occurs in the liver, pancreas and kidneys. From these organs it is transported in the blood for uptake by muscle. Despite synthesis taking place in other organs, Cr is found mainly in skeletal muscles and to a lesser extent in cardiac and smooth muscle. Skeletal muscle Cr concentrations depend on muscle fibre type (Edstrom et al., 1992, Tesch et al., 1989). The highest concentrations of Cr are found in fast-twitch (white) muscle. Both Cr and its precursors are found predominantly in meat, chicken and fish and Harris et al., (1992) have found lower than normal levels of Cr in the muscles of two vegetarians. However they seemed to be unaffected in daily life activities by their lower intramuscular Cr store (Harris et al., 1992).

An area of exercise physiology that has, until recently, received little interest is the effect of nutritional supplementation, using Cr, upon short-term high intensity exercise capacity. This is surprising given that many individual events (such as running, cycling and rowing) and almost all team sports, rely heavily on the 'high energy phosphate' pathways to provide energy for muscular contraction. Indeed, even athletes participating in endurance events of long duration intermittently increase their exercise intensity, particularly during the final 30 seconds of a race, and although PCr is not a primary fuel source, it may be of significant secondary importance.

Research on Cr supplementation in humans was begun as early as 1926, with studies focusing primarily on the way Cr is metabolised by the body (Chanutin, 1926; Crim et al., 1975) and on the size of the body Cr pool (Crim et al., 1976). The early studies did not require ingestion of large quantities of Cr and despite the fact that most of the body's Cr is stored in muscle, the investigators did not perform muscle biopsies. Therefore no conclusions could be made about the effect of supplementation on the intramuscular store of Cr.

But, a recent landmark study, which included muscle biopsies, showed that oral Cr supplementation of 20 grams of Cr per day for three days or more could significantly increase muscle Cr content (Harris et al., 1992),. This increased the size of the PCr pool available to rapidly provide fuel for ATP resynthesis (Harris et al., 1992).

2.3.2 CREATINE SUPPLEMENTATION AND EXERCISE PERFORMANCE

The study by Harris et al., (1992) prompted other studies that sought to examine the effect of the above supplementation regimen on exercise performance. Maximal, short duration exercise regimens were chosen since it was assumed that the major effect of Cr supplementation (and the resulting increased muscle PCr stores) would occur under these exercise conditions.

In summary, these studies have shown that Cr supplementation does not improve maximal exercise performance but does improve subjects' resistance to fatigue during short, intermittent, maximal exercise tests so that performance is enhanced during the later stages of the tests (Balsom et al., 1993a; Birch et al., 1994; Earnest et al., 1994 (Abstract); Greenhaff et al., 1993a; Lemon et al., 1995 (Abstract); Soderlund et al., 1994 (Abstract); Viru and Nurmeviki, 1993). The exercise protocols varied and included high intensity cycle ergometry involving 10 repetitions of 6 s maximal sprints with 30 s rest (Balsom et al., 1993a), 3 sets of 30 s maximal isokinetic sprint cycle bouts with 4 minutes rest between bouts (Birch et al., 1994), 3 sets of 30 s maximal cycle sprints with 5 minutes rest between bouts (Earnest et al., 1995 (Abstract)), 5 sets of 30 repetitions of maximal isokinetic knee extensions on a cybex with 1 minute rest between sets (Greenhaff et al., 1993a), 20-30 s of maximal isometric ankle extensions with 16 s rest between sets (Lemon et al., 1995 (Abstract)), 5 sets of 6 s cycle sprints with 30 s rest between sets and 1 set of 10 s cycle sprint with 40 s rest prior to the bout (Soderlund et al., 1994 (Abstract)) and running time trials of 4 repetitions of 300 m and 1 km with 3-4 minutes rest respectively between repetitions (Viru and Nurmekivi, 1993). It should be noted that of these studies only two (Greenhaff et al., 1993a and Lemon et al., 1995 (Abstract)) performed muscle biopsies and therefore determined the muscle Cr concentration before and after supplementation.

However, more recent research does not seem to concur. Preliminary reports of studies by Almada et al., (1995) (Abstract); Burke et al., (1995) (Abstract); Cooke et al., (1995); Grindstaff et al., (1995) (Abstract); Odland et al., (1994) (Abstract); Redondo et al., (1995) (Abstract) and Stroud et al., (1994) have indicated that Cr supplementation does not enhance performance under a variety of exercise conditions. These conditions include maximal bench-press and squat (Almada et al., 1995 (Abstract); Grindstaff et al., 1995 (Abstract)), swim sprints up to 100 m (Burke et al., 1995 (Abstract)), 2 sets of 15 s maximal cycling with 20 minutes rest between sets (Cooke et al., 1995), one maximal 30 s cycle sprint (Odland et al., 1995 (Abstract)), multiple 60 m sprints

(Redondo et al., 1995 (Abstract)) and a submaximal incremental run at different workloads adjusted every 6 minutes (Stroud et al., 1994). It is unknown whether or not the subjects actually Cr loaded since muscle biopsies were not performed and without this data it is difficult to determine the reasons for the unchanged performances. These protocols were not markedly diffeent from those in which performance improvements were noted.

2.3.3 CREATINE SUPPLEMENTATION AND CARBOHYDRATE METABOLISM

Cr and its effect on carbohydrate metabolism has indirectly been studied through the measurement of plasma pH and plasma lactate during exercise, before and after Cr loading. Conflicting evidence has been reported. Several studies (Balsom et al., 1993b; Birch et al., 1994; Greenhaff et al., 1993a; Stroud et al., 1994) have reported no change in blood lactate levels in either the placebo or Cr group following supplementation, suggesting that the administration of Cr does not affect the utilisation of the non-oxidative glycolytic and glycogenolytic process for providing energy for contraction. This was found for exercise modalities ranging from repeated bouts of maximal exercise lasting a total of approximately 1 minute (Balsom et al., 1993a) to submaximal incremental exercise lasting a total of approximately 42 minutes (Stroud et al., 1994). Studies by Balsom et al., (1993a) and Soderlund et al., (1994)(Abstract) showed that when performing high-intensity intermittent exercise lasting 6 s, those individuals who were supplemented with Cr had lower plasma lactate levels. These groups of researchers postulated that under Cr loaded conditions, the processes of non-oxidative glycolysis and glycogenolysis (and thus the production of lactate and hydrogen ions) will be utilised to a lesser extent during very short exercise bouts simply because the larger store of PCr is providing energy for a greater proportion of the exercise bout.

While the initial studies have provided some insight into the effects of the supplementation regimen on exercise performance, the performances that have been measured are generally of a short duration and supramaximal intensity. The effects of Cr supplementation on performance and metabolism described in these studies are applicable to only that type of exercise and the results cannot be extrapolated to other types of exercise. However, we hypothesise that higher PCr levels may result in less allosteric activation of the enzymes involved in non-oxidative glycolysis and glycogenolysis during exercise of longer duration. The result of this will be a lesser demand on glycolytic and glycogenolytic processes early on in the exercise bout, and thus lower plasma

lactate levels and possibly less of a decrease in intramuscular pH. In addition, oxidative phosphorylation plays a major role when in exercise, such as endurance exercise, the processes are linked with the ATP being produced by the mitochondria (as a result of oxidative breakdown of glucose, glycogen, fat or protein). This ATP is then transported to the contractile proteins of the myofibril via the 'Cr-PCr shuttle' and this may be more efficient with higher levels of TCr resulting in greater oxidative energy supply.

2.3.4 CREATINE SUPPLEMENTATION AND THE PURINE NUCLEOTIDE CYCLE

The initial studies on Cr supplementation while examing the effect of Cr on performance also measured plasma NH_3 (Birch et al., 1994; Greenhaff et al., 1993a) and Hx (Balsom et al., 1993a; Balsom et al., 1993b) levels as markers of adenine nucleotide degradation. However, plasma NH_3 is merely a marker of the extent of activity of AMP deaminase which deaminates AMP to IMP and thus indicates the extent of adenine nucleotide degradation to IMP (see 2.1.4). The use of other markers such as Hx and Ur aid in determining the extent to which the cycle as a whole has been functioning, with plasma Ur being an end marker of complete loss of adenine nucleotide from the cell. For this reason Ur could be viewed as an indicator of the degree to which *de novo* synthesis of adenine nucleotides needs to occur to subsequently replenish these lost adenine nucleotides.

Greenhaff et al., (1993a) found that oral Cr supplementation lowered plasma NH_3 levels post-supplementation during 5 sets of 30 repetitions of maximal isokinetic knee extensions with 1 minute rest between sets. Birch et al., (1994) found lowered plasma NH_3 following 3 sets of 30 s cycle sprints with 4 minutes rest between sets. Similarly the results of Balsom et al., (1993a) show that plasma Hx accumulation is decreased following Cr supplementation. As stated in the previous section, it is not possible to extrapolate results from maximal, short duration exercising situations to exercising conditions lasting one-hour. Balsom et al., (1993b) found no change in plasma Hx levels following a 6 km terrain run. Thus, a plasma NH_3 lowering effect may not occur during endurance exercise. In summary, the majority of the results of these initial studies have indicated that Cr supplementation can decrease the activity of the PNC during the later bouts of short-duration, maximal exercise or maximal intermittent exercise. This suggests more efficient maintenance of the ATP:ADP ratio, thence less activation of the PNC and less adenine nucleotide degradation when Cr supplemented. This effect may be due to either greater PCr availability or faster PCr resynthesis or both.

2.3.5 CREATINE SUPPLEMENTATION AND PHOSPHOCREATINE RESYNTHESIS

The effect of oral Cr supplementation on PCr resynthesis during recovery from intense, exhaustive exercise has been investigated and two studies indicate that PCr resynthesis is improved when Cr loaded (Greenhaff et al., 1994a; Greenhaff et al., 1993b). These two studies used electrical stimulation to induce isometric contraction of the quadriceps. The exercise involved 20 contractions lasting 1.6 s with 1.6 s rest. The tests were performed with and without occlusion of blood flow and pre and post Cr supplementation. These studies found an accelerated rate of PCr resynthesis after Cr supplementation especially in those individuals with the lowest levels of TCr prior to supplementation. This may explain the mechanism behind the performance improvements found in the latter bouts of exercise in several of the Cr supplementation studies described previously. Accelerated PCr resynthesis can result in an enhanced potential to perform maximally during subsequent exercise because there is a larger amount of high-energy phosphate available for transfer to ADP for resynthesis of ATP and less reliance on non-oxidative glycolysis with consequently less hydrogen ion production and better force maintenance.

2.4 CREATINE SUPPLEMENTATION AND ONE HOUR EXERCISE

As mentioned previously, Cr has another biochemical function which is to shuttle 'high-energy' phosphate formed during oxidative phosphorylation from the mitochondria to the working muscle proteins. During sub-maximal intensity exercise it is not expected that PCr breakdown itself would contribute substantially to the energy requirements, but that the glycogenolytic-glycolytic systems and oxidative phosphorylation would be the major contributors. Indeed, oxidative phosphorylation could contribute between 40 and 70% of the energy requirements depending on the intensity and the duration of the exercise (Astrand and Rodahl, 1970; Hawley and Hopkins, 1995). Thus, a possible benefit of increased muscle Cr stores could be an improved capacity to transport 'high-energy' phosphate in the form of PCr from the mitochondria to the contractile proteins where the 'high-energy' phosphate is utilised to rephosphorylate ADP to ATP thereby increasing the contribution of oxidative processes to energy requirements and decreasing the utilisation of non-oxidative glycogenolysis and glycolysis and delaying the accumulation of fatigue-inducing metabolites. To date no performance tests longer than 24 minutes of running have been performed (Balsom et al., 1993b), although CHO metabolism has been studied for 42 minutes at

submaximal exercise intensities (Stroud et al., 1994) with subjects who have been Cr supplemented. Thus the potential of Cr as an ergogenic aid for endurance athletes warrants further investigation.

During metabolically stressful situations a sub-optimal ATP:ADP ratio results in ADP degradation to AMP and inosine monophosphate (IMP) (Lowenstein, 1990; Sahlin and Broberg, 1990; Sewell and Harris, 1992; Tullson and Terjung, 1991) and later to Hx, X and Ur (Hellsten-Westing et al., 1994; Sutton et al., 1980). This results in a loss of adenine nucleotide from the muscle pool, but does maintain the ATP:ADP ratio for a period thereby allowing muscular activity to continue. No study has investigated whether an increased availability of PCr, which facilitates the resynthesis of ATP from ADP and PCr, will decrease ADP breakdown and adenine nucleotide loss from the muscle pool during endurance exercise and whether this will translate into improved performance. However indirect evidence from plasma NH_3 and Hx levels suggest that this may be the case as these plasma markers of adenine nucleotide loss are decreased following Cr supplementation (Balsom et al., 1993a; Birch et al., 1994; Greenhaff et al., 1993a).

Despite the integrated nature of metabolic control during exercise and the role of Cr in several processes, no research has examined the effects of creatine supplementation on performance or metabolism during one-hour endurance exercise. We therefore proposed to test the effects of creatine supplementation on performance and metabolism during a distance trial consisting of one-hour near maximal endurance cycling effort. Metabolic investigations included plasma lactate, NH_3, Hx and Ur; and muscle ATP, ADP, AMP, PCr, Cr and glycogen. The mechanism of action of creatine under these conditions was proposed to be an effect on the efficiency of the 'Cr-PCr shuttle' resulting in alterations in adenine nucleotide metabolism. We hypothesised that Cr loading would result in maintainance of PCr at higher levels during sub-maximal exercise and improved rephosphorylation of ATP so that the blood based indicators of adenine nucleotide catabolism would be lowered. We also hypothesised that performance would improve which would indicate that the ATP:ADP ratio is being more closely regulated because of the greater availability of Cr, consequently the onset of fatigue as a result of an accumulation of ADP and other fatigue-inducing metabolites would be delayed.

CHAPTER 3: METHODS AND MATERIALS

CHAPTER 3: METHODS AND MATERIALS

3.1 SUBJECTS

Twenty male endurance cyclists volunteered for the study. They were recruited on the basis of their ability to cycle approximately 40 kilometres in one hour. In addition they were required to have covered a distance of 105 kilometres in a cycle race in under 3 hours, and at the time of the study they should be participating in regular endurance training. The subjects received an information sheet outlining the procedures for the test period (Appendix 1) and were then required to sign an informed consent form (Appendix 2). In addition, they understood that they were able to withdraw from the study at any time if they so desired. The study was passed with ethical approval by the University of Cape Town Ethical Research Committee for research in human subjects.

3.2 EXPERIMENTAL PROCEDURE

This study was a placebo controlled, double-blind laboratory experiment to determine the effect of Cr ingestion on endurance exercise performance and metabolism. At no stage were either the investigator or the subjects aware of the nature of the tablets being ingested and both were unblinded only after analysis of all samples. The 2 week test period consisted of an initial week of familiarisation of the subjects with the exercise tests. Initially, each subject was required to perform a Peak Sustained Power Output (PPO) test (see 3.3 for detail) in order to assess their level of fitness and therefore their suitability for the trial. Two days later the subject was required to bring his own bicycle into the laboratory for attachment to a wind-braked cycle ergometer (Kingcycle, Version 4.1, 1991, EDS Portaprompt Limited, High Wycombe, Bucks). The subject then completed a one-hour distance cycle. This was performed under identical conditions to those under which the subjects were to perform during the trial period. This required that the subject only receive water to consume during the one-hour cycle and was unaware of distance covered, speed and power output. They were aware only of the length of time they had been cycling. Following this each subject was required to record his training for the familiarisation week prior to Test 1 (T1) and his diet for the three days prior to T1. Each subject was then asked to maintain the same training and diet for the second week of the trial, prior to Test 2 (T2).

Prior to T1 each subject was required to undergo a muscle biopsy (see 3.5 for detail) and the insertion of an in-dwelling cannula so that venous blood could be sampled at various time points (see 3.7 for detail). The subject then performed the one hour cycle during which he was to cover as much distance as possible. Total distance covered and heart-rate (Polar Vantage XL, Polar Electro, Finland) were recorded. Following the cycle each subject was given a bag containing either the placebo (supplied by LMN, England) or the Cr supplement (Ergomax, LMN, England). These tablets were exactly the same size, texture and weight. They were then instructed to consume 5 tablets four times per day for the following week and to record the time of day the tablets were taken and how they felt physically and psychologically after taking them. In addition they were instructed to dissolve the tablets in hot water, to minimize the conversion of Cr to creatinine, and to take each batch of five tablets before a main meal and the fourth batch either one hour before training or before going to sleep on a rest day.

Upon completion of the Cr loading week the subjects again reported to the laboratory and underwent a second muscle biopsy and insertion of a cannula. They again completed a one hour distance cycle (T2) to compare the effect of the loading week on performance and metabolism during the one-hour test.

3.3 THE PEAK POWER OUTPUT (PPO) TEST

This test is a routine laboratory test and was performed according to the protocol of Hawley and Noakes (1992). The test has been validated and is a reliable and consistent measure of the maximal sustained power output that can be performed by the athlete. A close correlation has been reported between peak workload achieved and VO_{2max} using this protocol ($r=0.97$, $p<0.0001$).

The exercise test is performed on a calibrated cycle ergometer (Lode, Groningen, Nederland). The starting workload is that workload which is determined as 3.33 Watts (W) per kilogram (kg) of the subject's weight. This workload is then increased every 2.5 minutes by 50 W for the initial 2 workloads and thereafter by 25 W until voluntary exhaustion of the subject or where the subject fails to maintain a cadence of greater than 70 revolutions per minute (rpm). The time of cessation of the test is recorded and the peak sustained power achieved by the subject is calculated according to the formula:

$$\text{Peak Power Output (PPO)} = \left(\frac{\text{time at final workload} * 25\ (W)}{150\ \text{sec}}\right) + \text{previous workload (W)}$$

The resulting PPO may also be used to predict VO_{2max} using the equation:

$$VO_{2max} = (0.01141\ (PPO) + 0.435)$$

In addition, the subjects' power to weight ratio can be calculated using the formula:

$$P{:}W = \frac{PPO\ (W)}{\text{mass (Kg)}}$$

This allows for comparison between individuals of different mass and thus takes into account the relative power that each individual produces rather than simply the absolute power produced by the cyclist which takes no account of body mass or size.

3.4 THE KINGCYCLE

The subjects cycled on their own bicycles during the familiarisation, T1 and T2 one hour cycles. Their bicycles were attached to the 'Kingcycle' ergometer (Version 4.1, 1991, EDS Portaprompt Limited, High Wycombe, Bucks.). This system involves the attachment of the front bicycle forks to a frame and the resting of the bottom bracket on an adjustable prop. Adjusting the prop alters the amount of rolling resistance applied to the rear wheel. Power is produced through the rolling of the rear tyre against a calibrated air brake and flywheel. The mass of the flywheel and the resistance of the air brake are engineered to simulate the inertial load experienced by a 65 kg cyclist and his bicycle. Cadence and power output are measured by a system of photo optic cells. The whole system is linked to an IBM compatible PC and the data is recorded and displayed continuously. This system has proven reliability (coefficient of variation = 1.0 ± 0.5 % for three 40 km time trials , Palmer et al., 1995), and results of maximal aerobic power tests performed on the Kingcycle indicate a test retest correlation of r=0.98 (Keen et al., 1991).

Prior to each test the Kingcycle was calibrated for each cyclist on his bicycle utilising a run down calibration. Calibration is achieved by altering the rolling resistance by manually altering the adjustable prop. This serves to ensure the results of the tests performed on the Kingcycle are both comparable and repeatable. The advantage of this system is that it allows the cyclists to be tested under conditions that more closely approximate road cycling and allows the cyclists to use their own bicycles.

3.5 MUSCLE SAMPLING

Muscle biopsy samples were taken on two occasions from the vastus lateralis muscle using the technique designed by Bergstrom et al. (1962) as modified by Evans et al. (1982). Some of the subjects (n = 10) did however, consent to having a third post-exercise biopsy. The muscle biopsies were taken under sterile conditions and performed by a qualified medical doctor with experience in this procedure. Prior to incision the skin was cleaned and prepared for administration of local anaesthetic. Following this approximately 3 ml of lignocaine (2% m/v Lignocaine, Intramed, Port Elizabeth, R.S.A.) was injected below the skin to the level of the muscle fascia. Once sufficient time had passed to allow the anaesthetic to act, a scalpel incision was made through the skin and fascia to the level of the muscle. The muscle biopsy needle was then inserted and a sample taken within 2 seconds of insertion. The muscle sample was then placed in a vial and frozen in liquid nitrogen and subsequently stored at -80°C. The time from taking of the biopsy to freezing was estimated to be 6 seconds. Pressure was then applied to the wound until the bleeding stopped, the biopsy wound was then disinfected with Spray Benzco (Beige Pharmaceuticals, Edenvale, R.S.A.), closed using Steristrip skin closures (3M, St. Paul, U.S.A), covered with Tegaderm (3M, Canada Inc., Ontario) and bandaged.

The frozen muscle sample was later freeze-dried and used for analysis of Cr, PCr, ATP, ADP, AMP and glycogen contents. Thus, the muscle samples were saved and pre and post samples were analysed together at a later date. The method used for determination of the high-energy phosphates and nucleotides was a modification of the High Performance Liquid Chromatography (HPLC) method used by Sellevold et al., (1986). Muscle glycogen content was analysed by a modified method of Keppler and Decker in Bergmeyer (ed), 1981.

3.6 HPLC METHOD FOR DETERMINATION OF MUSCLE NUCLEOTIDE CONTENTS

This method is based on that described by Sellevold et al., (1986). Certain modifications were made to provide better resolution of peaks in our apparatus. The equipment used for analysis included a Gilson Instruments HPLC (Gilson Inc., Middleton, U.S.A) using a Gilson 305 pump (Gilson Inc., Middleton, U.S.A), a Rheodyne 7125 injection valve (Rheodyne, California, U.S.A) and Gilson UV/ VIS detector (119UV) (Gilson Inc., Middleton, U.S.A). The column used was an Alphasil 5ODS 25 cm x 4.6 mm. The flow rate was set at 0.6 ml/ min from 0 to 8 minutes,

thereafter increased to 1.2 ml/ min until 19 minutes whereupon it was again reduced to 0.6 ml/ min. The total run time was 20 minutes. The injection loop consisted of 20 µl of sample.

The method used consisted of a mobile phase of phosphate buffer made up using 90 mM potassium dihydrogen phosphate (BDH, Hypersolv, England), 3.5 % HPLC-grade acetonitrile (BDH, Hypersolv, England) and 2,3 mM tetrabutylammonium hydrogensulphate (TBAHS) (TCI-Ace, Tokyo Kasei Kogyo Co. Ltd., Tokyo). The buffer was then titrated to pH 6.5 using 40 % KOH and subsequently made up to volume using distilled, 18 Mohm water. Following this the buffer was filtered and degassed.

Standards were made up using mobile phase buffer and were divided into aliquots and frozen at -20°C. Fresh standards were defrosted every morning and mixed together to form a combination of all the compounds expected in a muscle sample run. Thus, a high and a low standard mix were made up for injection onto the column. Standard runs were repeated at regular intervals during an analysis day.

On any analysis day, a freeze-dried muscle sample was dissected free of connective tissue (and in some samples blood) and approximately 5 mg dry wt weighed out. This sample was then homogenised in a glass homogeniser using 100 µl of ice-cold 0.6 M perchloric acid (PCA) (BDH, Hypersolv, England) with a further 900 µl being added susequently to make the volume up to 1 ml. Homogenisation was continued until the muscle sample was well homogenised. The sample was then placed in a 1.5 ml conical reaction vial and centrifuged in a micro-centrifuge (Eppendorf, Geratebau, Germany) for 4 minutes at 11500 rpm (8000g). Two 200 µl aliquots of supernatant were removed and placed in two reaction vials. In one vial 4 µl of universal indicator was added and titrated, with approximately 21 µl of 2.5 M K_2CO_3 (UnivAR, Saarchem Pty Ltd, Krugersdorp) being added to neutralise the sample. Following this the same procedure was performed on the second vial without the addition of universal indicator. This vial was then placed on ice for 10 minutes to precipitate the potassium perchlorate, and then centrifuged for 4 minutes at 11500 rpm. After centrifugation 100 µl of supernatant was removed and made up to 1 ml in another reaction vial using 900 µl mobile phase buffer. After mixing, approximately 20 µl was removed from the vial and injected onto the column via the injection valve.

This modified method was found to produce good peak resolution of all compounds investigated (see Appendix 3). These compounds were Cr, PCr, ATP, ADP, AMP. These peaks were quantified relative to the average areas and heights of the standards which were recorded at the start and end of each day. In particular, Cr was analysed according to height at 210 nm while ATP was analysed according to area at 260 nm.

3.7 BLOOD SAMPLING

Blood samples were taken at regular intervals during T1 and T2. They were drawn through an 18 gauge cannula and stopcock inserted into a forearm vein. The cannula was kept patent throughout the trial by regular flushings with sterile saline (0.9 % sodium chloride, Sabax, Johannesburg, R.S.A.) containing 1 ml of heparin (Novo Nordisk, Johannesburg, R.S.A.) per 100 ml saline. A blood sample was taken at rest and then at 10, 20, 40 and 60 minutes of the one-hour cycle and at 2, 6 and 10 minutes of recovery. Prior to taking each sample, 1.5 ml of blood was drawn and discarded. Following this approximately 3.5 ml of blood was drawn and placed on ice in 5 ml evacuated tubes (Vac-u-test, Radem Medical, Sandton, R.S.A.) containing 15 % K_3EDTA. This blood was then centrifuged (Sigma, GmbH, Germany) at 3000 rpm for 6 minutes at 3°C. Immediately after separation, 2 aliquots of 120 µl of plasma were removed from each sample and placed in a microcuvette (Halb-Mikro, Labortechnik, Greiner, Germany) and covered with parafilm for determination of plasma NH_3 (within 40 minutes of sampling) using a commercially available kit (NH_3, Boehringer Mannheim, Germany). The remaining plasma was removed and placed in plastic 1.5 ml storage vials and stored at -25°C for later determination of lactate, Hx and Ur. The samples were therefore saved and pre and post samples were analysed together at a later date. Lactate was analysed using a commercially available kit (Lactate PAP, Biomerieux, France), while Hx and Ur were analysed by HPLC.

3.8 HPLC METHOD FOR DETERMINATION OF HYPOXANTHINE AND URATE

Plasma Hx and Ur were analysed using a Gilson Instruments HPLC (Gilson Inc., Middleton, U.S.A) using a Gilson 305 pump (Gilson Inc., Middleton, U.S.A), a Rheodyne 7125 injection valve (Rheodyne, California, U.S.A) and Gilson UV/ VIS detector (119UV) (Gilson Inc., Middleton, U.S.A). The column used was an Alphasil 5ODS 25cm x 4.6mm. This analysis method utilised a 50 mM potassium dihydrogen phosphate buffer (BDH, Hypersolv, England) made up using distilled 18 Mohm water. Following this the buffer was filtered and degassed. The flow rate

was set at 1ml/ min and the total run time was 10 minutes. Integration of peaks was only begun after 5 minutes of the run. Stock standards were made up and frozen. These were defrosted at the start of every day and made up to volume with mobile phase buffer. Extraction of the plasma samples was similar to that for muscle samples. This method involved adding 100 µl of plasma to 100 µl of ice-cold 0.6 M perchloric acid (PCA) (BDH, Hypersolv, England). The sample was centrifuged in a micro-centrifuge (Eppendorf, Geratebau, Germany) for 4 minutes at 11500 rpm (8000g). Following this 150 µl of supernatant was removed and neutralised with 9 µl of 2.5 M K_2CO_3 (UnivAR, Saarchem Pty Ltd, Krugersdorp, R.S.A). This sample was then placed on ice for 10 minutes and again centrifuged for 4 minutes at 11500 rpm. After centrifugation 100 µl of supernatant was drawn off and made up to 500 µl with 400 µl mobile phase buffer. This sample was then injected onto the column. The injection volume was 20µl of sample.

Both Hx and Ur were detected at 249 nm and analysis was performed according to area. This method provided good resolution of peaks in both standard and sample runs (see Appendix 4).

3.9 TRAINING AND DIETARY RECORDS

The subjects were all required to keep accurate log-books of their training sessions including distance, type of course, time taken and intensity (on a scale of 1 to 10) of the session during their 2 weeks of testing. Their training in the initial familiarisation week was to be kept constant in the following week and this was recorded in a log-book In addition, the subjects were instructed to keep dietary records for the 3 days prior to T1. The food that was consumed on those 3 days was then to be (as closely as possible) consumed on the 3 days prior to T2 so that carbohydrate-loading and differing dietary practices between T1 and T2 could largely be eliminated. The rationale for requesting these records was to closely control for an effect of diet thereby limiting the effect on performance and metabolism to that of the creatine supplement or placebo.

3.10 STATISTICAL ANALYSIS

All statistical analysis was performed on Statistica (Statsoft Inc., Tulsa, Oaklahoma). For all statistical analysis of data on muscle compounds a two-way ANOVA with a repeated measure was used, while for all blood metabolites and heart-rate, a three-way ANOVA with repeated measures was used. Missing variables were deleted pairwise. Statistical significance was accepted when $p < 0.05$. Where significant differences were found a Tukey HSD post-hoc test for unequal numbers was performed to determine where significant differences lay.

CHAPTER 4 : THE EFFECT OF ORAL CREATINE SUPPLEMENTATION ON ONE-HOUR CYCLING PERFORMANCE AND METABOLISM

CHAPTER 4 : THE EFFECT OF ORAL CREATINE SUPPLEMENTATION ON ONE-HOUR CYCLING PERFORMANCE AND METABOLISM

4.1 RESULTS

All results are expressed as means and standard errors of the mean (± SEM) for each group at each time point. Statistical analyses which are graphically displayed as significant are only the between group differences. Differences in plasma metabolite concentrations from rest were statistically significant at all time points during exercise and recovery.

4.1.1 SUBJECT CHARACTERISTICS

There were no differences between the two groups for either age, height, mass or PPO, although both groups significantly increased their mass from T1 to T2 ($p<0.01$) (see Table I).

4.1.2 MUSCLE DATA

Both groups significantly increased their muscle Cr from T1 to T2 ($p<0.05$) (see Figure 3a). The initial level of muscle Cr for the placebo and Cr group was 49.8 ± 2.0 mmol/ kg dry wt and 51.1 ± 5.0 mmol/ kg dry wt respectively. This increased to 54.6 ± 3.7 mmol/ kg dry wt in the placebo group and 67.6 ± 4.4 mmol/ kg dry wt in the Cr group following supplementation. Figure 3b indicates that muscle PCr did not significantly change in either group from T1 to T2. Total muscle Cr (TCr, the sum of Cr and PCr) showed a significant effect of Cr loading ($p<0.001$) (see Figure 4). The Cr group increased their muscle TCr from 123.5 ± 3.8 mmol/ kg dry wt to 159.8 ± 7.9 mmol/ kg dry wt. This represents a mean increase of 29%. The placebo group was relatively unchanged from 126.7 ± 4.7 mmol/ kg dry wt to 127.5 ± 3.6 mmol/ kg dry wt. Figure 5a indicates the relationship between initial TCr and the degree to which Cr loading occurs. The relationship was not significant ($r=0.46$; $p=0.19$).

No significant differences were found for ATP, ADP and total nucleotide (the sum of ATP and ADP since AMP concentrations were negligible) contents in any of these variables indicating that the two groups were similar and that Cr loading did not affect the intramuscular nucleotide levels

(see Table II). In addition, in those subjects with large enough biopsy samples for both T1 and T2, further analysis for muscle glycogen was performed. No significant differences were noted in terms of glycogen loading or utilisation in either the placebo (n=4) or the Cr group (n=6) (see Table III).

4.1.3 PERFORMANCE AND HEART-RATE DURING THE ONE-HOUR CYCLE

The distance covered in the one-hour trial at T1 was not different between the Cr and placebo groups. The Cr group marginally improved the distance covered in the one-hour cycling trial (39.1 ± 0.9 km to 39.8 ± 0.8 km) while the placebo group was unchanged (39.3 ± 0.8 km to 39.2 ± 1.1 km). Figure 5b shows the correlation between the change in TCr and the change in distance covered in the creatine group. This relationship approaches significance ($r=0.6$, $p=0.06$), indicating that there is some relationship between the change in TCr levels and the degree to which perfomance improves. No significant differences were evident in the heart-rate data in or between either group for T1 or T2.

4.1.4 TRAINING AND DIETARY RECORDS

No significant differences were evident in the subjects training regimen before or during the Cr supplementation regimen. Similarly, the subjects reported the same or very similar dietary practices over the two week trial period and especially in the 24 hours prior to each trial.

4.1.5 BLOOD DATA

Figure 6a, b and c and Figure 7 display the concentrations of plasma NH_3, Hx, Ur and lactate during the one-hour trials and 10 minutes of recovery. For absolute values of mean ± SEM for each group at each time point see Appendix 5. It should be noted that although the T1 values for plasma NH_3, Hx and Ur in the Cr group were higher, these were not significantly different. Plasma NH_3 (see Figure 6a) was significantly different between groups, trials and over time ($p<0.05$). This indicates that the Cr and placebo groups responded differently from T1 to T2 at several time points. Post-hoc analysis indicated where the differences were.

Figure 6b displays the results of plasma Hx analysis. A significant difference ($p<0.01$) between the groups, trials and over time was noted indicating that the groups behaved differently from T1 to T2 at several time points. The exact location of these differences as determined through post-hoc

analysis are indicated on the respective graphs. The results of Ur analysis indicate a significant difference between T1 and T2 ($p<0.01$) when all the results are analysed together, indicating that both groups decreased. However, there were no differences between groups or between trials in each group separately (see Figure 6c). It is for this reason that the significant difference is not indicated on Figure 6c. Plasma lactate results are displayed in Figure 7. Statistical analysis revealed no significant differences in or between either group in T1 and T2.

4.2 DISCUSSION

The main finding of this study was that increases in intramuscular Cr stores were associated with decreased plasma NH_3 and Hx concentrations during maximal one-hour exercise in comparison to the unloaded state and to controls who ingested placebo. This has only previously been found in studies examining the effect of Cr supplementation on metabolism during short-term maximal exercise (Balsom et al., 1993a; Birch et al., 1994; Greenhaff et al., 1993a). The alteration in metabolism in this study was not associated with an improvement in one-hour cycling performance and was not a result of alterations in dietary practices or training by the subjects participating in the study.

The exercise performed in this study was of a high intensity but continued for one hour. During this type of exercise, energy is provided by several systems including the oxidative and non-oxidative pathways, pathways of adenine nucleotide metabolism and 'immediate-energy' pathways involving the transfer of 'high-energy' phosphate. This is in contrast to the majority of previous studies that have to a large extent examined the effect of a similar Cr loading regimen, first described by Harris et al., (1992), on high intensity activity of short duration (Balsom et al., 1993a; Birch et al., 1994; Cooke et al., 1995; Earnest et al., 1994 (Abstract); Greenhaff et al., 1993a; Lemon et al., 1995 (Abstract); Soderlund et al., 1994 (Abstract)).

These studies showed some degree of performance improvement following Cr supplementation. Two other studies have examined the effect of Cr supplementation on relatively long oxygen-requiring exercise lasting up to a total of 16 minutes (Viru and Nuremviki, 1993) and 24 minutes (Balsom et al., 1993b) but no studies have examined the effect of oral Cr supplementation on

longer duration endurance exercise such as examined in this study. Viru and Nuremviki (1993) found that performance was improved in both 300 m and 1000 m run times as well as in total run time of 4 x 300 m and 4 x 100 m. However, Balsom et al., (1993b) showed no improvement in performance following Cr supplementation on a 6 km terrain run. We showed no improvement in exercise performance lasting one hour. This is unlikely to be due to insensitivity of our exercise test, since our laboratory has previously shown a low coefficient of variation for 40 km time-trials (coefficient of variation = 1.0 ± 0.5 %,) lasting an average of 56:24 ± 3:59 minutes (Palmer et al., 1995).

Nevertheless, despite a lack of effect on performance, this study showed that Cr loading alters metabolism during one-hour exercise and recovery following the one-hour cycle. This was shown by the changes in indicators of adenine nucleotide metabolism. The indicators of adenine nucleotide metabolism examined in this study included plasma ammonia (NH_3), hypoxanthine (Hx) and urate (Ur) concentrations. Previous studies have shown that plasma NH_3 levels are lowered during supramaximal exercise when Cr supplemented (Birch et al., 1994; Greenhaff et al., 1993a). Another study has shown a lowering of plasma Hx levels during 10 bouts of 6 s short-term high intensity exercise (Balsom et al., 1993a), while Balsom et al., (1993b) showed no change in plasma Hx levels after Cr supplementation during a 6 km terrain run. In this study, plasma and Hx were lowered in the Cr group following supplementation. It should be noted that the plasma levels of these two products were elevated (non-significantly) in the Cr group prior to supplementation. This is unlikely to be due to diet or training as these were kept consistent from one week to the next, and were not elevated to a similar degree following supplementation.

The purine nucleotide cycle (PNC) is generally believed to operate only under high intensity exercise conditions when the balance between ATP supply and ADP phosphorylation is no longer possible and there is a continuing high energy demand. Under these circumstances the myosin ATPase reaction restores ATP and produces AMP which is immediately deaminated to produce IMP and a further series of catabolic reactions ensues. The aim of these reactions is to decrease the level of ADP, in order to maintain an optimal ATP:ADP ratio such that contraction can continue. A result of this is that adenine nucleotide is lost from the muscle pool. The amount of 'lost' nucleotide is evidenced in the levels of plasma NH_3, Hx and Ur. Each of these metabolites

indicate the degree to which the PNC has been in operation and they are produced at various levels in the PNC.

It should be noted at this point that protein catabolism is also a source of NH_3 during exercise. MacLean et al., (1991) have shown that NH_3 production during exercise lasting approimately 100 minutes is predominantely derived from protein catabolism. However, Lemon and Mullin (1980) have previously shown that protein catabolism occurs primarily under conditions where subjects are either fasted or carbohydrate (CHO) depleted prior to the exercise tests. In the current study the subjects were neither fasted or CHO depleted prior to the exercise, nor did the exercise last up to 100 minutes. In addition, the only change between T1 and T2 was an increase in TCr. Thus, only the pathway of NH_3 production, affected by the increase in TCr, could possibly have been affected. This indicates that even during high-intensity submaximal exercise lasting one hour, a significant proportion of NH_3 produced is most likely from AMP deamination.

Thus, Cr loading results in more efficient maintenance of the ATP:ADP ratio with less activation of the PNC indicated in the lowering of the plasma NH_3 and Hx levels post supplementation. We hypothesise that more 'high energy' phosphate may be delivered to the myofibrils through the 'Cr-PCr shuttle' due to the increased muscle total Cr (TCr) levels after loading. This hypothesis is also discussed in studies by Birch et al., (1994); Cooke et al., (1995); Greenhaff et al., (1993a); Greenhaff et al., (1993b); Greenhaff et al., (1994b); Greenhaff, (1995) and Viru and Nurmeviki, (1993). The increase in TCr necessarily increases the amount of PCr, thus increased amounts of 'high energy' phosphate are available for ADP rephosphorylation. In keeping with increased rates of ADP rephosphorylation will be a decrease in the level of AMP and subsequently less IMP (and therefore NH_3), less Hx and less Ur. Thus, the increased provision of 'high energy' phosphate (from PCr) available for ADP rephosphorylation has probably resulted in the lessening of adenine nucleotide degradation seen in this study. This is likely to involve both the mitochondrial and myofibrillar sites of the 'Cr-PCr shuttle' since the rephosphorylation of Cr is reliant on ATP formed during oxidative processes and occurs at the mitochondrial membrane, and the rephosphorylation of ADP is likely to be at the myofibrillar site of utilisation. This is analogous to the faster resynthesis rate previously shown at rest during recovery from exhaustive short-term high intensity exercise bouts (Greenhaff et al., 1993b; Greenhaff et al., 1994b). However, we

provide the first evidence that this is also a significant effect of Cr loading during high intensity endurance exercise.

It has recently been discovered that successive days of high-intensity training (Hellsten-Westing et al., 1993; Stathis et al., 1994) result in prolonged decreases in the resting levels of adenine nucleotide in muscle. Although the exercise protocol used in the present study was not similar to those high intensity training protocols, it still activated the PNC to a fairly large degree. This is evidenced in the plasma NH_3, Hx and Ur levels. The improved maintenance of the ATP:ADP ratio by the 'Cr-PCr shuttle' rather than through the activation of the PNC ultimately results in improved maintenance of intramuscular adenine nucleotides and less subsequent nucleotide salvage and less *de novo* ATP synthesis which is energetically expensive (Tullson and Terjung, 1991).

Several studies using supramaximal workloads (Balsom et al., 1993a; Balsom et al., 1993b; Birch et al., 1994; Greenhaff et al., 1993a; Soderlund et al., 1994 (Abstract); Stroud et al., 1994) have also examined the effect of Cr loading on carbohydrate metabolism by measuring plasma lactate levels during exercise. Balsom et al., (1993a) and Soderlund et al., (1994) (Abstract) showing a lowering of plasma lactate levels when Cr supplemented, but, other studies (Balsom et al., 1993b; Birch et al., 1994; Greenhaff et al., 1993a; Stroud et al., 1994) showing no change in plasma lactate levels pre- and post-supplementation. These apparently discrepant results can be explained by the different natures of the exercise protocols used. In those cases where the exercise duration is sufficiently short that an elevated resting PCr could supply substantial anaerobic energy, plasma lactate level is likely to be reduced. However, when the length of the exercise bout is increased and the proportion of anaerobic energy supplied by glycolysis overwhelms that supplied by PCr, then any effect of Cr supplementation on lactate concentration appears to be negligible.

The results of this study show that plasma lactate levels were relatively unchanged during a one-hour distance trial following Cr supplementation and this serves to confirm those results found by both Balsom et al., (1993b); Birch et al., (1994); Greenhaff et al., (1993a) and Stroud et al., (1994). The fact that plasma lactate levels are unchanged implies that there has been no alteration in non-oxidative carbohydrate (CHO) metabolism. It is more likely that the degree of activation of non-oxidative CHO metabolism is directly related to cycling intensity. This is supported by the

unchanged total cycling distance and the similar heart-rate results from T1 to T2. There were also no changes after Cr supplementation in exercise intensity at any particular time during the trials.

The effect of Cr loading on muscle glycogen loading and utilisation was examined in several subjects. Cr loading did not significantly affect the degree of muscle glycogen loading and the relative utilisation of muscle glycogen was similar between the two trials. The Cr group had higher (non-significant) muscle glycogen stores initially but utilised glycogen at a faster absolute rate, rendering the relative utilisation rate almost exactly the same. Bosch et al., (1993) have previously shown that those individuals with higher muscle glycogen stores use more glycogen during endurance exercise. However, the relative amount of muscle glycogen utilised by the 2 groups in this trial was similar. These results indicate that a tendancy exists towards Cr supplementation increasing muscle glycogen concentration as well as its subsequent absolute utilisation rate. Indeed, the results may have been more significant had more subjects consented to a third muscle biopsy.

In the light of these results, it would appear that Cr supplementation has no effect on CHO metabolism during one hour of high intensity exercise, a finding that has been described in several studies examining maximal exercise. Any effect previously shown is probably the result of the duration of the exercise bout. However, the area of Cr supplementation and its effect on muscle glycogen concentration and utilisation is one requiring further investigation and therefore this discussion does not imply that there was any effect of the Cr supplement.

The Cr loading regimen described first by Harris et al., (1992) significantly increased intramuscular Cr levels by 20 %, however only two subsequent studies have confirmed this finding (Greenhaff et al., 1993; Lemon et al., 1995 (Abstract)). In this study, it was found that well-trained endurance cyclists increased their intramuscular TCr stores by an average of 36.3 mmol/ kg dry wt or 29 %. However, Cr increased significantly in both the placebo and Cr groups in this study. This finding may be the result of a delay in freezing times or an artefact of the freezing process (Bangsbo et al., 1993). Several other studies on Cr loading have not performed muscle biopsies and thus have made conclusions about the effect of Cr loading on exercise performance, simply assuming that Cr loading had occured (Balsom et al., 1993a; Birch et al., 1994; Earnest et al., 1994 (Abstract); Greenhaff et al., 1993a; Viru and Nuremviki, 1993). However, closer examination of the

individual muscle biopsy results in this study show, like Harris et al., (1992) that there is considerable individual variability in the degree to which subjects Cr loaded. This was not related to the initial intramuscular TCr concentration ($r=0.46$, $p=0.19$). The latter finding is in contrast to the finding in Harris et al., (1992) where those individuals with the lowest TCr levels loaded to the largest extent.

Previous studies (summarised by Spriet, 1995) have indicated that the PCr content of Type I fibres has been found to be 5 - 15 % lower than in Type IIa and IIb. Although this is indicative of either a difference in storage capacity or a difference in uptake of Cr under unsupplemented conditions, our laboratory hypothesised that individual variation in the uptake or storage capacity (or both) of Cr supplements may also be influenced by fibre type. Myburgh et al., (Med. Sci. Sports Exerc., 1996 (Abstracts)) have recently shown that fibre type differences influenced Cr uptake with a strong positive correlation between percent Type IIb and the extent of change in TCr after a similar supplementation regimen and in similar calibre endurance-trained athletes ($r = 0.95$, $p < 0.005$) to those used in this study. This implies that Cr uptake may be lower in those athletes more suited to endurance activity than in those suited to sprinting (who characteristically have more Type IIb fibres). Thus, in this study, it is possible that of the sample of ten cyclists who were supplemented with Cr, some tended to be better sprinters or have more Type IIb than others, explaining the large individual variability in the results.

It is not known whether this influence of fibre type on Cr uptake following supplementation also exists in the more physiological state of lower circulating plasma Cr content. Should this be the case, it is possible that endurance trained athletes might have a lower baseline muscle TCr than sprinters. A comparison of the baseline data of this study with that of others shows that the mean level of TCr was normal. Therefore, I conclude that endurance trained athletes have a normal muscle TCr content prior to a Cr supplementation regimen, and elevate it to a similar degree as other studies (Harris et al., 1992; Greenhaff et al., 1993; Lemon et al., 1995 (Abstract)) following supplementation.

In this study only one individual responded to Cr loading by increasing intramuscular TCr by less than 10 mmol/ kg dry wt. This subject had an initial TCr of 108 mmol/ kg dry wt which increased to 117 mmol/ kg dry wt following one week of Cr loading. This is only an increase of 8 %. The

range of TCr reported in the literature (Harris et al., 1974) is 113.2 - 135.6 mmol/ kg dry wt. A maximal Cr loaded level of 191 mmol/ kg dry wt was found in one subject in this study which is in contrast to Harris et al., (1992) who stated that muscle Cr content appears to have an upper limit of 150 - 160 mmol/ kg dry wt and that this level cannot be exceeded. This result did not appear to be due to higher baseline levels which ranged from 108-147 mmol/ kg dry wt. The higher loading may be a result of exercising immediately after taking Cr throughout the loading week, a finding of Harris et al., (1992) shown in 5 subjects.

Regression analysis indicated that the degree of Cr loading is fairly well related to the improvement in performance ($r=0.6$, $p=0.06$), that is, those individuals who increase their TCr the most will be those individuals most likely to improve their performance in one hour of high-intensity cycling. The fact that one subject was a non-responder to Cr loading was examined as a possible reason for this close to but non-significant result. A recent study by Lemon et al., (1995) (Abstract) also described a non-responder. In this study, the elimination of the non-responder from analysis did little to alter the significance of this finding and for this study the Cr group was not divided into responders and non-responders. This is probably justified until further confirmation and analysis has been performed on prevalence of non-responders.

Another notable finding was that levels of nucleotide in the rested muscle biopsy samples did not change after Cr loading. The levels of nucleotide in resting muscle samples following Cr loading has only been reported in one study (Harris et al., 1992). This finding is not surprising given that the effect of Cr supplementation is largely related to its effect on the increase in potential energy for ATP resynthesis (that is , Cr and PCr) during contraction and it is during contraction and resynthesis during recovery that the effect of Cr loading has been reported (Balsom et al., 1993a; Birch et al., 1994; Cooke et al., 1995; Greenhaff et al., 1993a; Greenhaff et al., 1993b; Greenhaff et al., 1994a; Greenhaff et al., 1994b; Greenhaff, 1995; Viru and Nurmeviki, 1993).

Thus, while Cr loading may not affect the exercise performance of the type of exercise examined in this study, it may be that the cumulative effects of Cr loading and the lowering of the activity of the PNC may only become evident later on in exercise, for example, in a two hour exercise test. It may be under these circumstances that the effect of improved maintenance of the ATP:ADP ratio and less PNC activation and adenine nucleotide loss may result in the cyclist being better able to

maintain a high exercise intensity over a long exercise duration. Thus, improvements in performance may be noted in exercise lasting longer periods of time at similar intensities to those examined in this study where the effect of long-term activation of the PNC and maintainance of the ATP:ADP ratio in the face of decreasing adenine nucleotide levels may become relevant in terms of the development of fatigue.

4.3 CONCLUSIONS

Thus, oral Cr supplementation with 20 g of Cr per day for 7 days did not improve exercise performance during a one-hour distance cycle. However, the supplementation regimen increased TCr to a similar degree in endurance athletes as that found in previous studies. In addition, CHO metabolism was unaltered as evidenced by unchanged plasma lactate levels post-supplementation and similar muscle glycogen utilisation rates. However, plasma NH_3, Hx and Ur levels were lowered post-supplementation. This indicates that Cr supplementation has an effect on adenine nucleotide metabolism during a one-hour distance cycle.

CHAPTER 5: THESIS CONCLUSIONS

CHAPTER 5: THESIS CONCLUSIONS

The ultimate aim of Cr supplementation in athletes is to improve their exercise perfomance. However for performance improvement it is necessary for the body to alter its' metabolism or muscle function. An alteration in metabolism may result in improved energy production or less of an accumulation of fatigue-inducing metabolites or both. From these results it would seem that the effect of Cr supplementation during submaximal exercise is most likely an enhancement in PCr rephosphorylation and this is manifested as a alteration in adenine nucleotide metabolism and not in carbohydrate metabolism. However, any effect on performance was not apparent under the circumstances of the exercise tests in this study. It is highly likely that the changes in the metabolic variables may only result in an effect on performance as the exercise duration increases whilst maintaining a similar exercise intensity. Thus, a one hour cycle may not have been sufficient for the improved maintenance of the ATP:ADP ratio and therefore the lowering in adenine nucleotide compounds to have an effect on performance. This hypothesis is, in part, based on the slight increase in distance covered by the Cr group and the correlation between the change in TCr and the change in performance which indicated a tendancy ($p=0.06$) for peformance to improve the more a subject Cr loaded. This may have become significant had the exercise been continued for a longer period of time. Thus, a recommendation for future research is to perform a similar trial but for one and a half hours at a predetermined exercise intensity followed by a 20 km time trial. Alternatively an effect on performance during an exercise protocol such as used in this study may only become apparent under other conditions such as elevated temperature or carbohydrate depletion where ammonia production is already elevated.

In conclusion, the major effect of Cr supplementation has been shown to occur during the later bouts of intermittent, short duration, high intensity exercise. However, although Cr loading does not result in a performance improvement it does alter metabolism during one hour endurance exercise. Therefore, the effects of Cr supplementation on performance and metabolism during longer duration exercise is an area for future research.

TABLES

Table I: Subject characteristics for the placebo (n=10) and creatine (n=10) groups.

Group	Age (yrs)	Height (m)	Mass 1 (kg)	Mass 2 (kg)	PPO (W)
Placebo	23.64 ± 1.03	1.78 ± 0.03	74.65 ± 2.46	75.05 ± 2.5*	372.8 ± 13.6
Creatine	23.58 ± 0.92	1.79 ± 0.02	74.26 ± 2.81	75.08 ± 2.8*	365.8 ± 14.9

Values are expressed as means ± SEM. Mass 1 = pre-supplementation mass; Mass 2 = post-supplementation mass. Statistical analysis by 2-way ANOVA. * indicates significant difference ($p<0.05$) from Mass 1.

Table II: Muscle adenine nucleotide contents (mmol/ kg dry wt) in the placebo (n=10) and creatine (n=10) group for T1 and T2.

Group	ATP-T1	ATP-T2	ADP-T1	ADP-T2	TNuc-T1	TNuc-T2
Placebo	22.52±0.7	24.96±1.3	2.63±0.2	2.85±0.3	25.15±0.8	27.81±1.6
Creatine	23.97±0.7	25.42±1.1	2.68±0.1	2.60±0.2	26.65±0.7	28.02±1.2

Values are expressed as mean ± SEM. ATP = adenosine triphosphate, ADP = adenosine diphosphate, TNuc = Total nucleotide (ATP + ADP), T1 = pre-supplementation biopsy, T2 = post-supplementation biopsy. Statistical analysis by 2-way ANOVA.

Table III: Muscle glycogen concentrations (mmol/ kg dry wt) and utilisation (mmol/ kg dry wt/ hr) in both the placebo (n=4) and the Cr (n=6) groups pre- and post-supplementation and post -exercise.

Group	Test 1	Test 2	Post-Test 2	Utilisation	% utilised
Placebo	423 ± 31	407 ± 10	138 ± 11	269	66 %
Creatine	488 ± 59	554 ± 13	184 ± 6	370	67 %

Values are expressed as means ± SEM. Test 1 = pre-supplementation biopsy, Test 2 = post-supplementation biopsy, Post-Test 2 = post-supplementation post-exercise biopsy. Statistical analysis by 2-way ANOVA.

FIGURES

Figure 1: The components of action of the 'Cr-PCr shuttle' and the typical mean Cr and PCr concentrations in rested muscle.

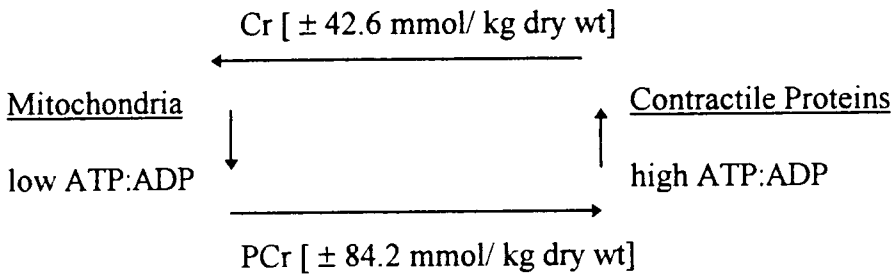


Figure 2: Diagrammatic representation of the pathways of purine nucleotide synthesis and degradation.

Underlined compounds are those markers of adenine nucleotide degradation measures in this study during exercise and recovery.

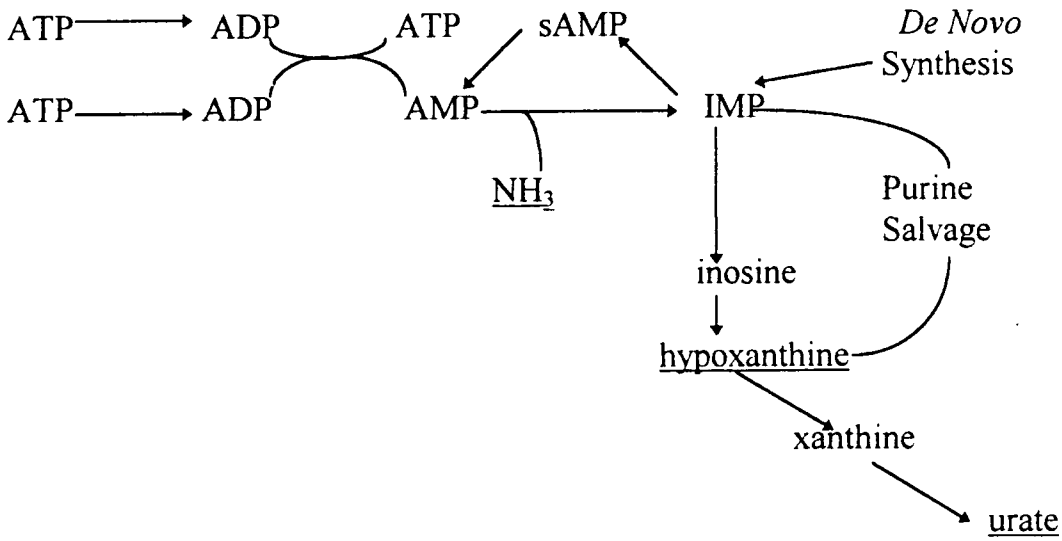
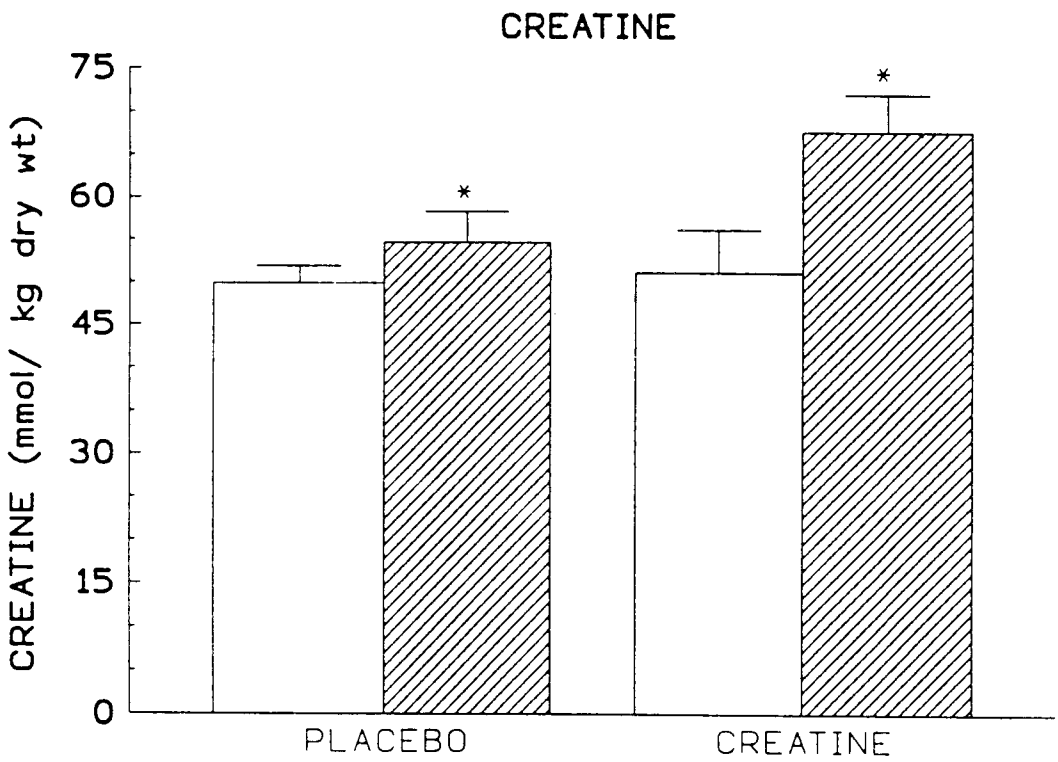


Figure 3a: Intramuscular creatine concentration (mean ± SEM) in both the placebo (n=10) and creatine (n=10) groups pre- and post- supplementation.
b: Intramuscular phosphocreatine concentration (mean ± SEM) in both the placebo (n=10) and creatine (n=10) groups pre- and post- supplementation.

Clear bar = pre-supplementation, hatched bar = post-supplementation. Statistical analysis by 2-way ANOVA. * indicates significant difference from pre-supplementation ($p<0.05$).

a)



b)

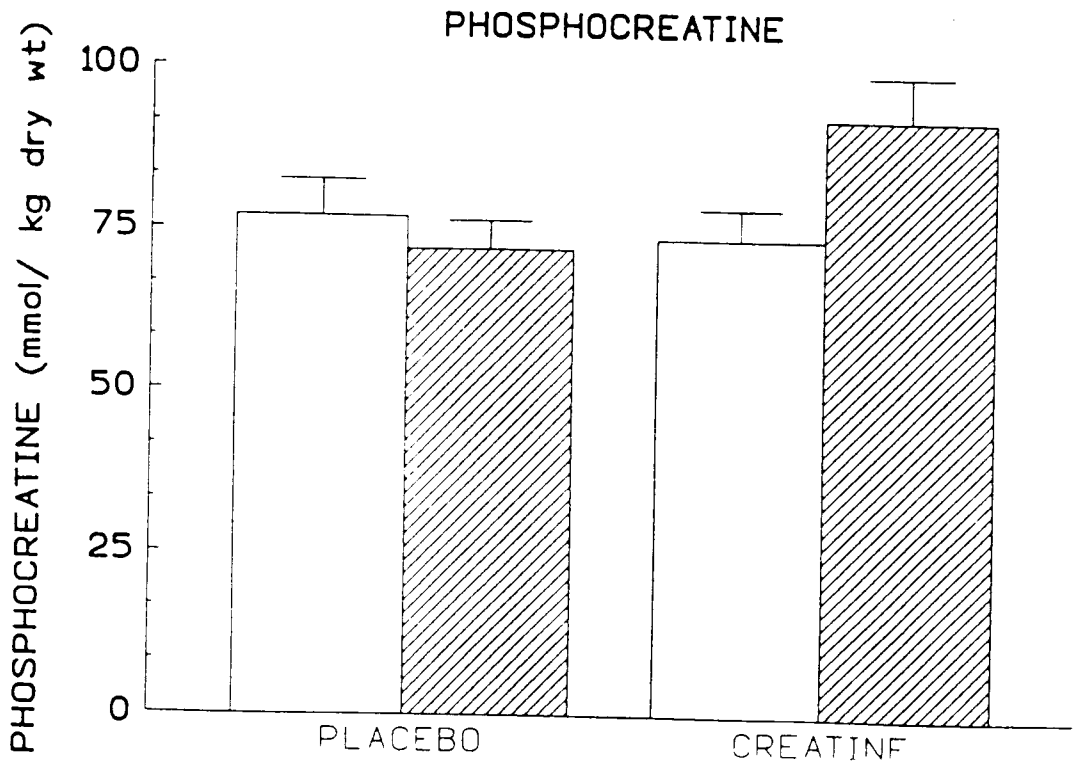


Figure 4: Intramuscular total creatine concentration (mean ± SEM) in both the placebo (n=10) and creatine (n=10) groups pre- and post- supplementation.

Clear bar = pre-supplementation, hatched bar = post-supplementation. Statistical analysis by 2-way ANOVA. * indicates significant difference from pre-supplementation ($p<0.05$).

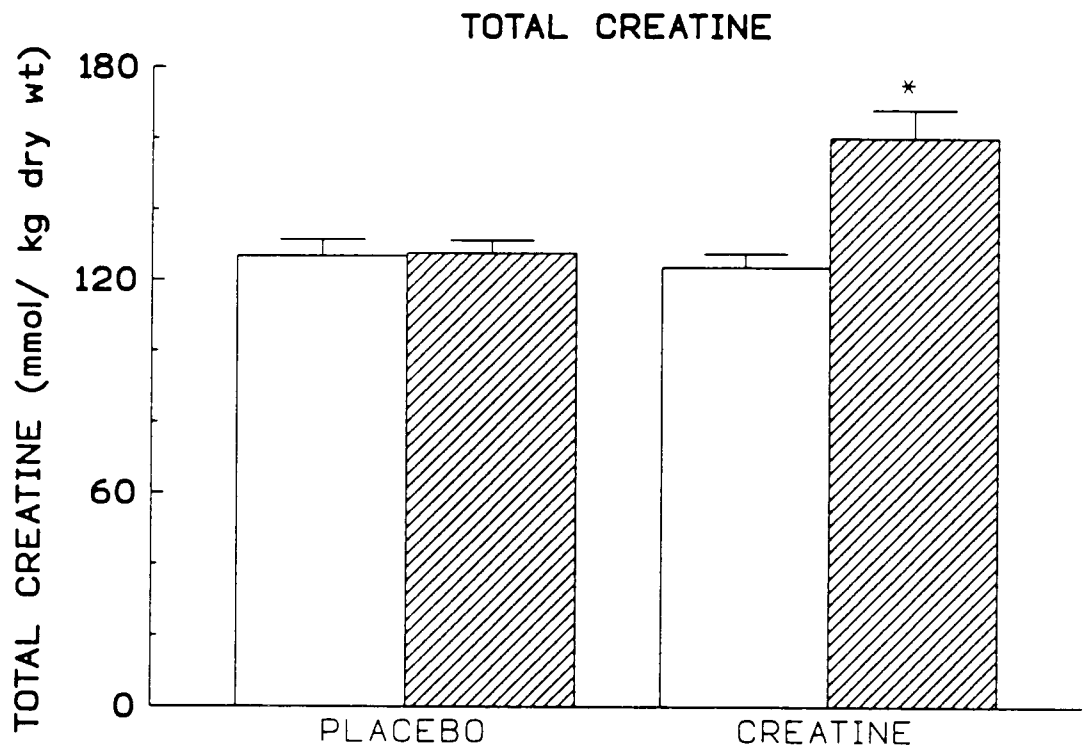
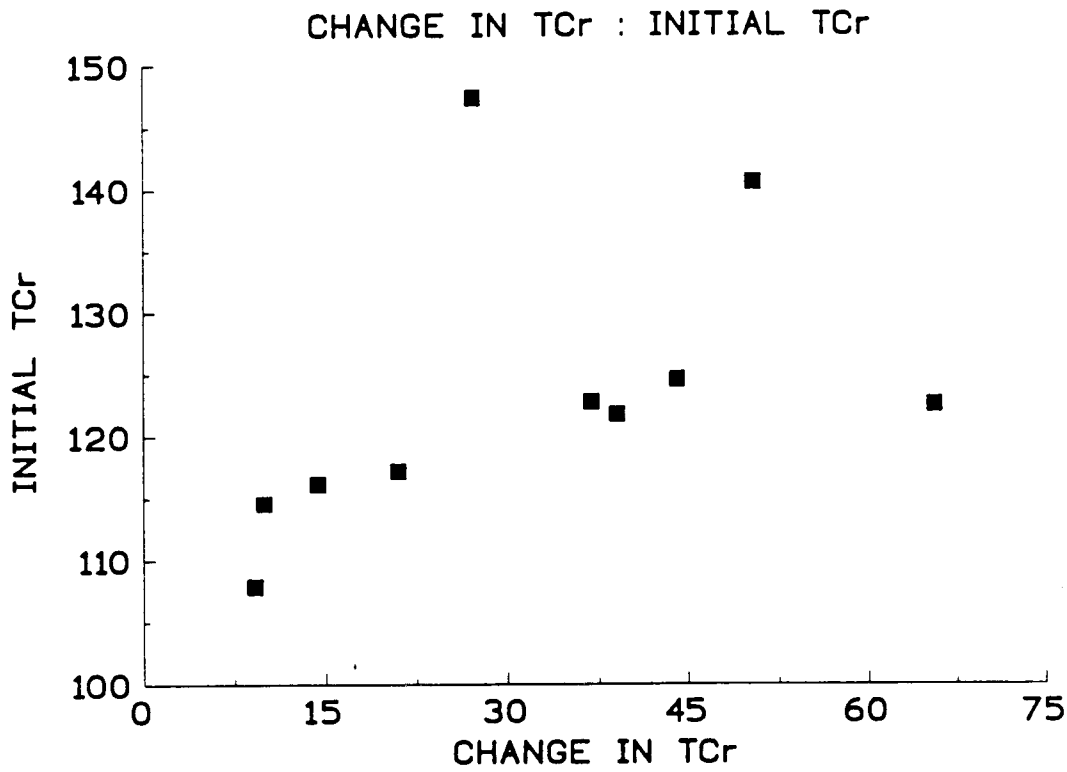


Figure 5a: The relationship between the initial total creatine concentration and the change in total creatine concentration in the creatine group (n=10) (r=0.46, p=0.19).
b: The relationship between the change in total creatine concentration and the change in the distance covered in the creatine group (n=10) (r=0.6, p=0.06).

a)



b)

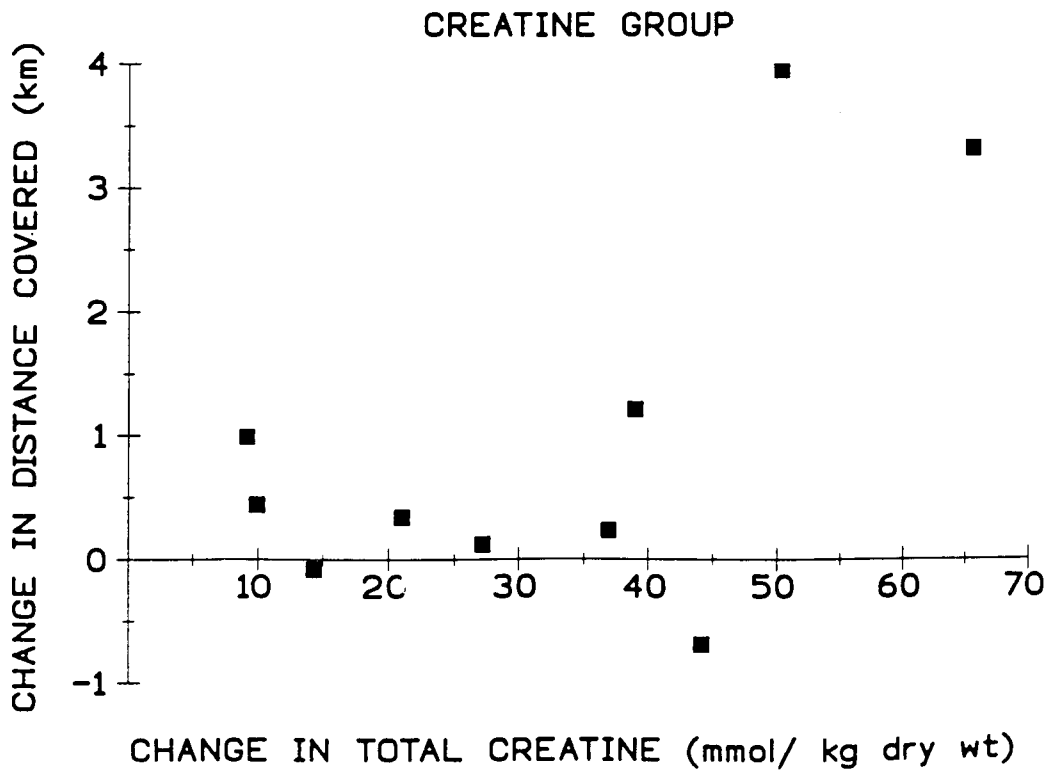


Figure 6a, b, c: Plasma ammonia, hypoxanthine and urate concentrations (mean ± SEM) in the creatine (n=10) and (n=10) placebo group during exercise (0 to 60 minutes) and recovery (up to 70 minutes) pre- and post- supplementation.

Open symbol = pre-supplementation, filled symbol = post-supplementation. Statistical analysis by 3-way ANOVA. * indicates significant difference from pre-supplementation level and from placebo group and from time 0 ($p<0.05$). Plasma urate results for all subjects (n=20) shows a significant difference but this is not indicated.

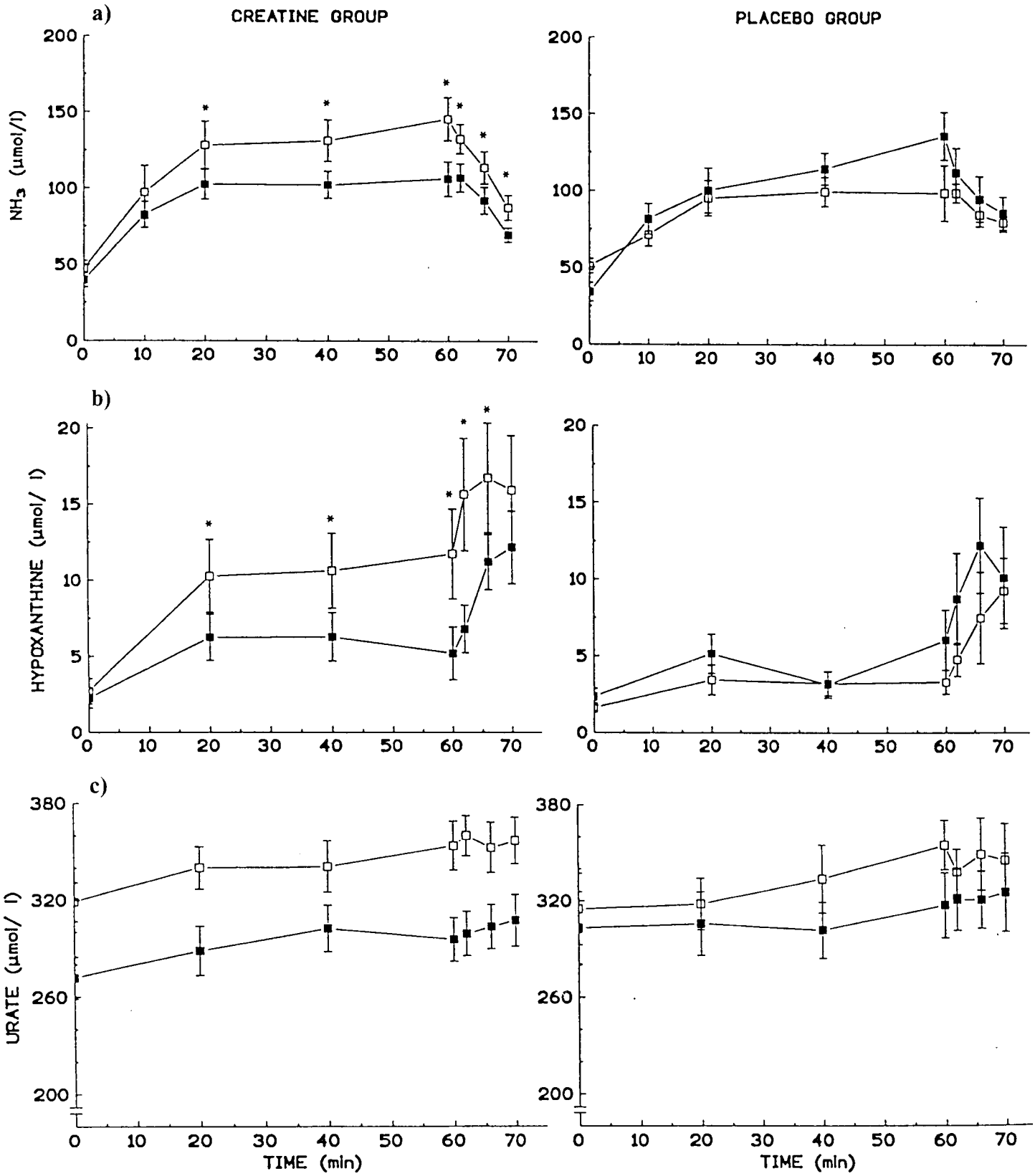
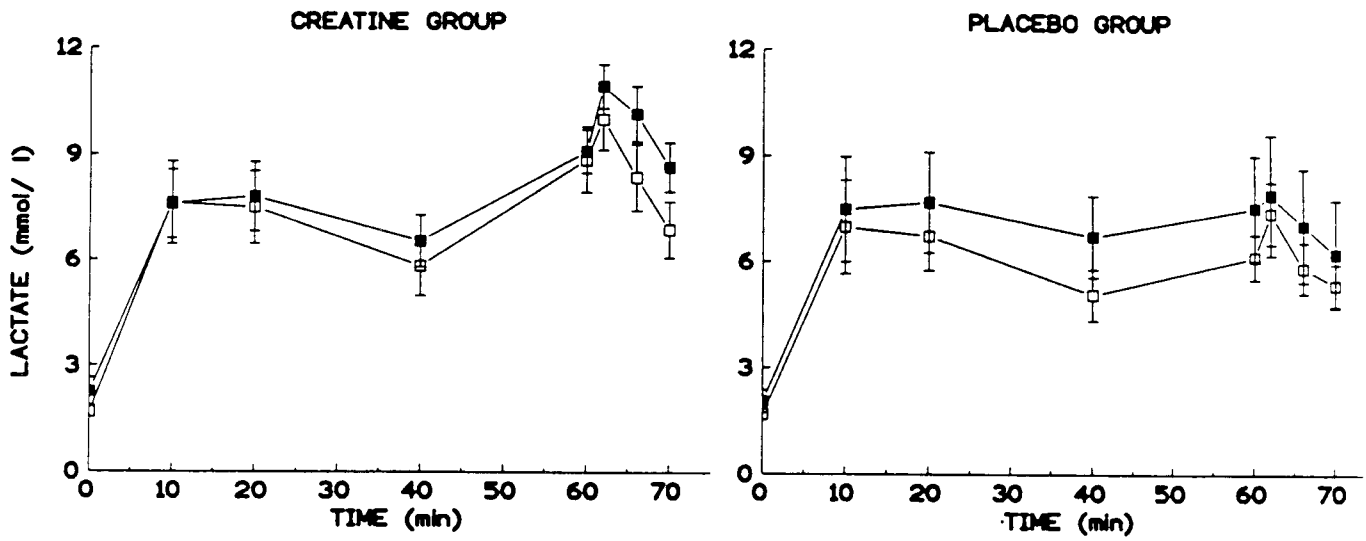


Figure 7: Plasma lactate concentration (mean ± SEM) in the creatine (n=10) and placebo (n=10) groups pre- and post- supplementation.

Open symbol = pre-supplementation, filled symbol = post-supplementation. Statistical analysis by 3-way ANOVA. Significant time effect from time 0 not indicated on graph.



REFERENCES

REFERENCES

Almada A, R Kreider, L Weiss, A Fry, L Wood, D Bullen, M Miyaji, P Grindstaff, L Ramsey and Y Li. Effects of ingesting a supplement containing creatine monohydrate for 28 days on isokinetic performance. Med. Sci. Sports Exerc. 27(5):S146, 1995 (Abstract).

Astrand P-O and K Rodahl. Textbook of work physiology. McGraw-Hill, USA, 1970.

Balsom PD, B Ekblom, K Soderlund, B Sjodin and E Hultman. Creatine supplementation and dynamic high-intensity intermittent exercise. Scand. J. Med. Sci. Sports 3:143-149, 1993a.

Balsom PD, SDR Harridge, K Soderlund, B Sjodin and B Ekblom. Creatine supplementation *per se* does not enhance endurance exercise performance. Acta Physiol. Scand. 149:521-523, 1993b.

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

Bangsbo J, B Sjodin and Y Hellsten-Westing. Exchange of hypoxanthine in muscle during intense exercise in man. Acta Physiol. Scand. 146:549-550, 1992.

Bangsbo J, B Johansen, B Quistorff and B Saltin. NMR and analytic biochemical evaluation of CrP and nucleotides in the human calf during muscle contraction. J. Appl. Physiol. 74(4):2034-2039, 1993.

Banister EW, W Rajendra and BJC Mutch. Ammonia as an indicator of exercise stress. Implications of recent findings to Sports Medicine. Sports Med. 2:34-46, 1985.

Banister EW and BJC Cameron. Exercise-induced hyperammonemia: Peripheral and central effects. Int. J. Sports Med. 11(2):S129-S142, 1990.

Bergstrom J. Muscle electrolytes in man: Determined by neutron activation analysis in needle biopsy specimens. A study on normal subjects, kidney patients, and patients with chronic diarrhoea. Scand. J. Clin. Lab. Invest. 14(Supplement 68), 1962.

Bessman SP and CL Carpenter. The creatine-creatine phosphate energy shuttle. Ann. Rev. Biochem. 54:831-862, 1985.

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

Bosch AN, SC Dennis and TD Noakes. Influence of carbohydrate loading on fuel substrate turnover and oxidation during prolonged exercise. J. Appl. Physiol. 74(4): 1921-1927, 1993.

Bouckaert J and JL Pannier. Blood ammonia response to treadmill and bicycle exercise in man. Int. J. Sports Med. 16:141-144, 1995.

Broberg S and K Sahlin. Adenine nucleotide degradation in human skeletal muscle during prolonged exercise. J. Appl. Physiol. 67(1):116-122, 1989.

Brouns F, E Beckers, AJM Wagenmakers and WHM Saris. Ammonia accumulation during highly intensive long-lasting cycling: individual observations. Int. J. Sports Med. 11(2):S78-S84, 1990.

Burke LM, DB Payne and RD Telford. Oral creatine supplementation does not improve sprint performance in elite swimmers. Med. Sci. Sports Exerc. 27(5):S146, 1995 (Abstract).

Chanutin A. The fate of creatine when administered to man. J Biol. Chem. 67:29-41, 1926.

Cooke R. The mechanism of muscle contraction. Crit. Rev. Biochem. 21(1):53-118, 1986.

Cooke R, K Franks, GB Luciani and E Pate. The inhibition of rabbit skeletal muscle contraction by hydrogen ion and phosphate. J. Physiol. Lond. 395:77-97, 1988.

Cooke WH, PW Grandjean and WS Barnes. Effect of oral creatine supplementation on power output and fatigue during bicycle ergometry. J. Appl. Physiol. 78(2):670-673, 1995.

Crim MC, DH Calloway and S Margen. Creatine metabolism in men: urinary creatine and creatinine excretion with creatine feeding. J. Nutr. 105:428-438, 1975.

Crim MC, DH Calloway and S Margen. Creatine metabolism in men: creatine pool size and turnover in relation to creatine intake. J. Nutr. 106:371-381, 1976.

de Haan A, DA Jones and AJ Sargeant. Changes in velocity of shortening, power output and relaxation rate during fatigue of rat medial gastrocnemius muscle. Pflugers Arch. 413:422-428, 1989.

Dudley GA and RL Terjung. Influence of aerobic metabolism on IMP accumulation in fast-twitch muscle. Am. J. Physiol. 248(Cell Physiol. 17):C37-C42, 1985a.

Dudley GA and RL Terjung. Influence of acidosis on AMP deaminase activity in contracting fast-twitch muscle. Am. J. Physiol. 248(Cell Physiol. 17):C43-C50, 1985b.

Earnest CP, PG Snell, TL Mitchell, R Rodriguez and AL Almada. Effect of creatine monohydrate ingestion on peak anaerobic power, capacity and fatigue index. Med. Sci. Sports Exerc. 26(Supplement 5):S39, 1994 (Abstract).

Edwards HT, R Margaria and DB Dill. Metabolic rate, blood sugar and the utilisation of carbohydrate. Am. J. Physiol. 108:203-209, 1934.

Edstrom L, E Hultman, K Sahlin and H Sjoholm. The contents of high-energy phosphates in different fibre types in skeletal muscles from rat, guinea-pig and man. J. Physiol. 332:47-58, 1982.

Evans WJ, SD Phinney and VR Young. Suction applied to muscle biopsy maximizes sample size. Med. Sci. Sports Exerc. 14:101-102, 1982.

Gaitanos GC, C Williams, LH Boobis and S Brooks. Human muscle metabolism during intermittent maximal exercise. J. Appl. Physiol. 75(2):712-719, 1993.

Graham TE, J Bangsbo, PD Gollnick, C Juel and B Saltin. Ammonia metabolism during intense dynamic exercise and recovery in humans. Am. J. Physiol. 259(Endocrinol. Metab. 22):E170-E176, 1990.

Greenhaff PL, A Casey, AH 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. 84:565-571, 1993a.

Greenhaff PL, K Bodin, RC Harris, E Hultman, DA Jones, DB McIntyre, K Soderlund and DL Turner. Influence of oral creatine supplementation on muscle phosphocreatine re synthesis following intense contraction in man. J. Physiol. 467:75P, 1993b.

Greenhaff PL, D Constantin-Teodosiu, A Casey and E Hultman. The effect of oral creatine supplementation on skeletal muscle ATP degradation during repeated bouts of maximal voluntary exercise in man. J. Physiol. 476.P:84, 1994a.

Greenhaff PL, K Bodin, K Soderlund and E Hultman. Effect of oral creatine supplementation on phosphocreatine resynthesis. Am. J. Physiol. E725-E730, 1994b.

Greenhaff PL. Creatine: its role in physical performance and fatigue and its application as a sports food supplement. Insider. 3(1):1-4, 1995.

Grindstaff P, R Kreider, L Weiss, A Fry, L Wood, D Bullen, M Miyaji, L Ramsey, Y Li and A Almada. Effects of ingesting a supplement containing creatine monohydrate for 7 days on isokinetic performance. Med. Sci. Sports Exerc. 27(5):S146, 1995 (Abstract).

Hargreaves M. Skeletal muscle carbohydrate metabolism during exercise. in Hargreaves M (ed). Exercise Metabolism.. Human Kinetics, Champaign, Illinois, 1995.

Harris RC, E Hultman and LO Nordesjo. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scand. J. Clin. Lab. Invest. 33:109-120, 1974.

Harris RC, RHT Edwards, E Hultman, LO Nordesjo, B Nylind and K Sahlin. The time course of phosphorylcreatine resynthesis during recovery of the quadriceps muscle in man. Pflugers Arch. 367:137-142, 1976.

Harris RC, K Soderlund and E Hultman. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clin. Sci. 83:367-374, 1992.

Hawley JA and TD Noakes. Peak power output predicts maximal oxygen uptake and performance time. Eur. J. Appl. Physiol. 65:79-83, 1992.

Hawley JA and WG Hopkins. Aerobic glycolytic and aerobic lipolytic power systems. A new paradigm with implications for endurance and ultraendurance events. Sports Med. 19(4):240-250, 1995.

Hellsten-Westing Y, PD Balsom, B Norman and B Sjodin. The effect of high-intensity training on purine metabolism in man. Acta Physiol. Scand. 149:405-412, 1993.

Hellsten-Westing Y, L Kaijser, B Ekblom and B Sjodin. Exchange of purines in human liver and skeletal muscle with short-term exhaustive exercise. Am. J. Physiol. 266(Regulatory Integrative Comp. Physiol. 35):R81-R86, 1994.

Jacobus WE. Respiratory control and the integration of heart high-energy phosphate metabolism by mitochondrial creatine kinase. Ann. Rev. Physiol. 47:707-25, 1985.

Katz A, S Broberg, K Sahlin and J Wahren. Muscle ammonia and amino acid metabolism during dynamic exercise in man. Clin. Physiol. 6:365-379, 1986.

Keen PS, L Passfield and T Hale. Indirect determination of VO_{2max} using a sports specific (cycling) ergometry system. J. Sports Sci. 9(4):420, 1991(Abstract).

Keppler D and K Decker. Glycogen: determination with amyloglucosidase. in Bergmeyer HU (ed). Methods of Enzymatic Analysis. Verlag Chieme International, Florida, 3:1127-1129, 1981.

Korge P and KB Campbell. The importance of ATPase microenvironment in muscle fatigue: A hypothesis. Int. J. Sports Med. 16:172-179, 1995.

Lemon PWR and JP Mullin. Effect of initial muscle glycogen levels on protein catabolism during exercise. J. Appl. Physiol. 48:624-629, 1980.

Lemon PWR, M Boska, D Bredle, M Rodgers, T Ziegenfuss and B Newcomer. Effect of oral creatine supplementation on energetics during repeated maximal muscle contraction. Med. Sci. Sports Exerc. 27(5):S204, 1995 (Abstract).

Lowenstein JM. The purine nucleotide cycle revised. Int. J. Sports Med. 11(2):S37-S46, 1990.

MacLean DA, LL Spriet, E Hultman and TE Graham. Plasma and muscle amino acid and ammonia responses during prolonged exercise in humans. J. Appl. Physiol. 70(5):2095-2103, 1991.

McArdle WD, FI Katch and VL Katch. Exercise Physiology: Energy, Nutrition and Human Performance. Lea and Febiger, Philadelphia, 1991.

Meyer RA and RL Terjung. AMP deamination and IMP reamination in working skeletal muscle. Am. J. Physiol. 239:C32-38, 1980.

Meyer RA, GA Dudley and RL Terjung. Ammonia and IMP in different skeletal muscle fibers after exercise in rats. J. Appl. Physiol. 1037-1041, 1980.

Meyer RA, TR Brown, BL Krilowicz and MJ Kushmerick. Phosphagen and intracellular pH changes during contraction of creatine-depleted rat muscle. Am. J. Physiol. 250(Cell Physiol.19):C264-C274, 1986.

Meyer RA and JM Foley. Testing models of respiratory control in skeletal muscle. Med. Sci. Sports Exerc. 26(1):52-57, 1994.

Myburgh KH, A Bold, B Bellinger, G Wilson and TD Noakes. Creatine supplementation and sprint training in cyclists: metabolic and performance effects. Med. Sci. Sports Exerc. 28:S81:A483, 1996 (Abstract).

Odland LM, JD MacDougall, M Tarnopolsky, A Elorriaga, A Borgmann and S Atkinson. The effect of oral creatine supplementation on muscle [PCr] and power output during a short-term maximal cycling task. Med. Sci. Sports Exerc. 26(Supplement 5)S23, 1994 (Abstract).

Palmer GS, SC Dennis, TD Noakes and JA Hawley. Assessment of the reproducibility of performance testing on an air-braked cycle ergometer. Int. J. Sports Med. In press, 1995.

Radda GK, J Odoom, G Kemp, DJ Taylor, C Thompson and P Styles. Assessment of mitochondrial function and control in normal and diseased states. Biochimica et Biophysica Acta. 1271:15-19, 1995.

Redondo D, M Williams, E Dowling, B Graham, S Jones and A Almada. The effect of oral creatine monohydrate supplementation on running velocity. Med. Sci. Sports Exerc. 27(5):S146, 1995 (Abstract).

Sahlin K and S Broberg. Adenine nucleotide depletion in human muscle during exercise: Causality and significance of AMP deamination. Int. J. Sports Med. 11:S62-S67, 1990.

Sellevold OFM, P Jynge and K Aarstad. High performance liquid chromatography: A rapid isocratic method for determination of creatine compounds and adenine nucleotides in myocardial tissue. J. Mol.Cell. Cardiol. 18:517-527, 1986.

Sewell DA and RC Harris. Adenine nucleotide degradation in the thoroughbred horse with increasing exercise duration. Eur. J. Appl. Physiol. 65:271-277, 1992.

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

Soderlund K, PD Balsom and B Ekblom. Creatine supplementation and high-intensity exercise: Influence on performance and muscle metabolism. Clin. Sci. 84(Supplement 196):120, 1994 (Abstract).

Spencer MK and A Katz. Role of glycogen in control of glycolysis and IMP formation in human muscle during exercise. Am. J. Physiol. 260 (Endocrinol. Metab. 23):E859-E864, 1991.

Spriet LL. Anaerobic metabolism during high-intensity exercise. in Hargreaves M (ed). Exercise Metabolism. Human Kinetics, Champaign, Illinois, 1995.

Stathis CG, MA Febbraio, MF Carey and RJ Snow. Influence of sprint training on human skeletal muscle purine nucleotide metabolism. J. Appl. Physiol. 76(4):1802-1809, 1994.

Stroud MA, D Holliman, D Bell, AL Green, IA MacDonald and PL Greenhaff. Effect of oral creatine supplementation on respiratory gas exchange and blood lactate accumulation during steady-state incremental exercise and recovery in man. Clin. Sci. 87:707-710, 1994.

Stryer L. Biochemistry. Fourth Edition. Freeman and Co., New York :447, 1995.

Sutton JR, CJ Toews, GR Ward and IH Fox. Purine metabolism during strenuous muscular exercise in man. Metabolism. 29(3):254-260, 1980.

Sweeney HL. The importance of the creatine kinase reaction : The concept of metabolic capacitence. Med. Sci. Sports Exerc. 26(1):30-36, 1994.

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

Thompson GH, GJ Kemp, AL Sanderson and GK Radda. Skeletal muscle mitochondrial function studied by kinetic analysis of postexercise phosphocreatine resynthesis. J. Appl. Physiol. 78(6):2131-2139, 1995.

Tullson PC, HB John-Adler, DA Hood and RL Terjung. De novo synthesis of adenine nucleotides in different skeletal muscle fiber types. Am. J. Physiol. 225(Cell Physiol.24):C271-C277, 1988.

Tullson PC, DM Whitlock and RL Terjung. Adenine nucleotide degradation in slow-twitch red muscle. Am. J. Physiol. 258(Cell Physiol. 27):C258-C265, 1990.

Tullson PC and RL Terjung. Adenine nucleotide degradation in striated muscle. Int. J. Sports Med. 11:S47-S55, 1990.

Tullson PC and RL Terjung. Adenine nucleotide metabolism in contracting skeletal muscle. in Holloszy JO(eds). Exerc. and Sport Sci. Reviews, Williams and Wilkins Baltimore, 19:507-537, 1991.

Urhausen A and W Kindermann. Blood ammonia and lactate concentrations during endurance exercise of differing intensities. Eur. J. Appl. Physiol. 65:209-214, 1992.

Viru M and A Nurmekivi. Action of creatine intake on performance capacity of middle-distance runners. Biological and Pedagogical Problems of Physical Education and Sport. III:31-36, 1993.

Wagenmakers AJM. Role of amino acids and ammonia in mechanisms of fatigue. in Marconnet P, PV Komi, B Saltin, OM Sejersted (eds): Muscle fatigue mechanisms in exercise and training. Med. Sport Sci. Basel, Karger. 34:69-86, 1992.

Walker JB. Creatine: Biosynthesis, regulation and function. Adv. Enzymol. 50:177-242, 1979.

Walliman T, M Wyss, D Brdiczka, K Nicolay and HM Eppenberger. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem. J. 281:21-40, 1992.

Wilson DF. Factors affecting the rate and energetics of mitochondrial oxidative phosphorylation. Med. Sci. Sports Exerc. 26(1):37-43, 1994.

Wyss M and T Walliman. Creatine metabolism and the consequences of creatine depletion in muscle. Mol. Cell Biochem. 133/134:51-57, 1994.

APPENDICES

APPENDICES

APPENDIX 1: INFORMED CONSENT FORM

INFORMED CONSENT

THE EFFECTS OF CREATINE SUPPLEMENTATION ON HIGH-INTENSITY ENDURANCE PERFORMANCE IN ENDURANCE-TRAINED ATHLETES

I, _____(print name please)
hereby agree to participate in a research project at the MRC/UCT Bioenergetics of Exercise Research Unit, University of Cape Town Medical School.

I understand that this project will involve 4 separate visits to the laboratory separated by one week each. The first visit will involve a cycle test to exhaustion with progressively increasing workloads ("max test"); the second visit will comprise a familiarisation ride on the King Cycle ergometer for 60 minutes, during which time I will cycle at the highest intensity which I can sustain for that time using my own bicycle placed on the ergometer. I agree to use a back tyre which has never been punctured for all this trial and the following two visits. I will receive instructions on how to complete a training and dietary intake diary.

The third visit will comprise a cycle performance test on the King Cycle ergometer in which I will cycle at the highest intensity which I can sustain for a period of 1 hour. Heart rate, speed and distance will be monitored. During this test blood samples will be taken from a catheter inserted into the forearm vein. Approximately 80 ml of blood will be taken. Prior to the exercise test, I will have the first muscle biopsy. I understand that a muscle biopsy is a minor surgical technique which will be performed by a medical doctor experienced in this technique. A local anaesthetic will be administered and the procedure will be performed under sterile conditions. As with any surgical technique there is a small risk of infection. However, no intramuscular infection has ever been described as a complication of a muscle biopsy. In our laboratory over the past 12 years we have performed approximately 1000 muscle biopsies. We have had 1 skin infection and 1 allergic response to the tape used to cover the incision.

I will be given tablets to take for the following 7 days. The tablets may be placebo (non-active agent) or active agent. I agree to take one tablet 4 times per day: three at meal times and 1 tablet one hour before a training session.

The fourth visit to the lab will comprise a pre-exercise biopsy, the same 1 hour distance trial followed immediately by a second muscle biopsy. At this time I will hand in my training and dietary records. I understand that the code of placebo vs active agent will only be broken after all subjects have completed the trial. In addition, no results of performance during the distance trial will be made available until all subjects have completed the trial.

I fully understand the risks associated with this project. Furthermore, I understand that I am free to withdraw from the study at any time should I so choose.

Signature _____

Date_____ Principal Investigator _____

APPENDIX 2: INFORMATION SHEET

THE EFFECTS OF CREATINE SUPPLEMENTATION ON HIGH-INTENSITY ENDURANCE PERFORMANCE IN ENDURANCE-TRAINED ATHLETES

Information sheet

Summary: This study is a 7-day placebo-controlled double-blind study to determine the effect of creatine loading (20g per day) on high intensity endurance performance for 1 hour in 20 well trained cyclists. In addition to exercise performance, blood samples and muscle biopsy samples will be analysed for serum acid-base balance, lactate, ammonia and hypoxanthine and muscle creatine, phosphocreatine, ATP and glycogen contents. A placebo-controlled double-blind study is one in which ten subjects receive the active agent (in this case creatine) and 10 receive a substitute which looks in all respects similar to the active agent. Neither the subject nor investigators are aware of whether the agent is active or placebo until after all analysis is completed.

Protocol:

Week 1: visit 1: Standard VO_2max test;
visit 2: familiarisation with the King cycle = 1 hour trial;
training (7-day) and dietary (3-day) record sheets.

Week 2: pre-exercise muscle biopsy;
exercise test: 1 hour trial at highest possible intensity
randomised administration of placebo or active agent (creatine tablets): 4 x 5g/day for 7 consecutive days;
repeat and record similar training and diet.

Week 3: pre-exercise muscle biopsy; exercise test 2; post-exercise muscle biopsy

Exercise Tests: all to be performed with an unpunctured rear tyre
*1 hour distance trial performed with the subjects own bicycle placed on a King Cycle (which measures distance achieved; speed and average power output). Blood sampling every 10 min for 50 min, then every 5 min for 10 min and during recovery. This is approximately 80 ml.
*VO_2 max test performed on a laboratory bicycle starting at an intensity of 3.3 W/kg body mass and increased by 50 W for 150 sec and then by 25 W/150 sec.

Muscle biopsy:Payment:R100 x 3 and R150 bonus on completion of all biopsies.
A muscle biopsy is a minor surgical technique which will be performed by a medical doctor experienced in this technique. A local anaesthetic will be administered by a very small needle.

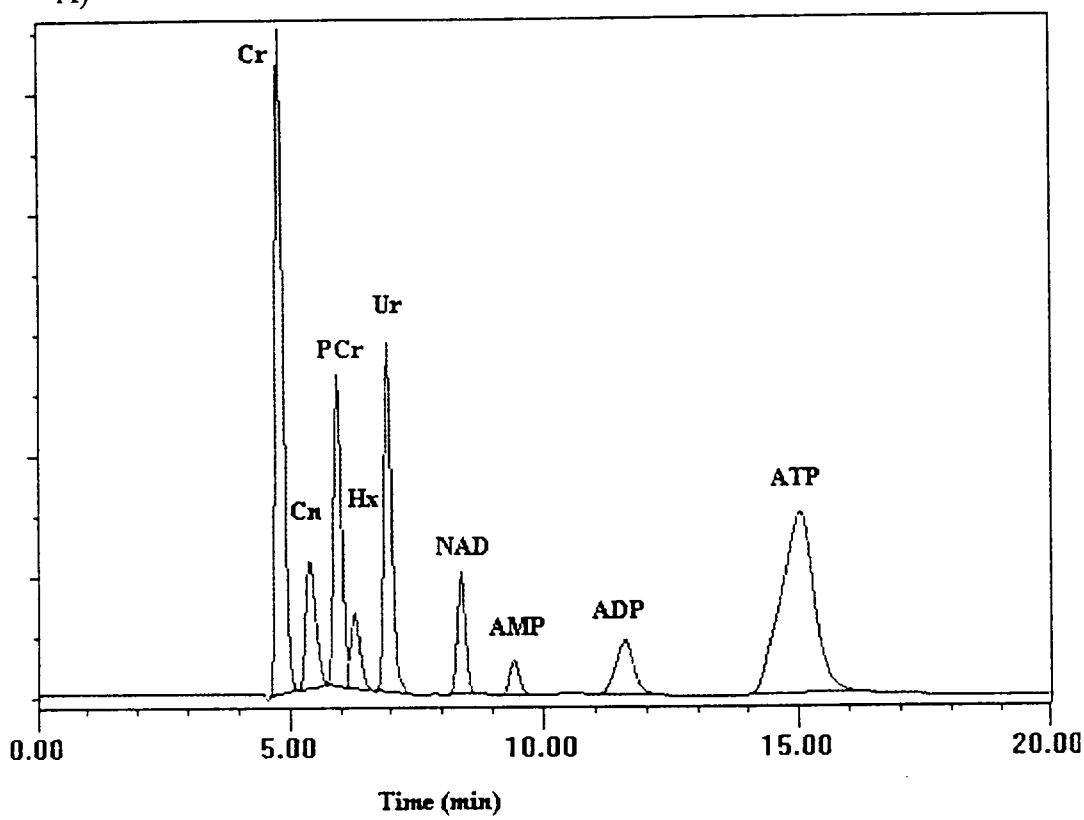
The muscle biopsy is performed with a needle which has a 6mm diameter. The needle is sterilised at Grootte Schuur hospital and the biopsy is performed under sterile conditions. There is a minimal risk of infection. Training will not be affected by the biopsy.

Tablets:
You will be given 20 effervecent tablets/day to take for the following 7 days. The tablets may be placebo (non-active agent) or active agent. They should be dissolved in luke-warm water or tea/coffee. they are tasteless. 5 tablets should be taken at each of 3 meal times and 5 tablets one hour before a training session. If you have a rest day, take the fourth batch of tablets before bed.

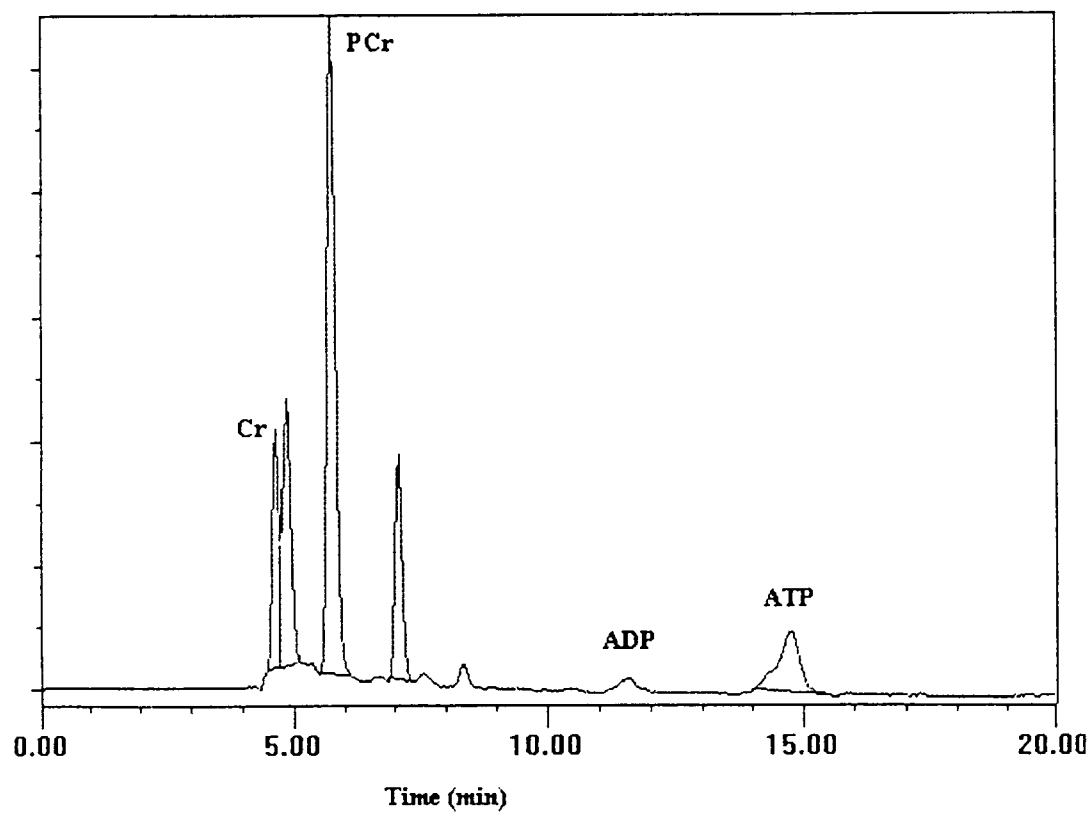
PROBLEMS: Brett 4394699

APPENDIX 3: EXAMPLE OF A CHROMATOGRAM FOR BOTH A) A STANDARD SOLUTION AND B) A MUSCLE SAMPLE

A)

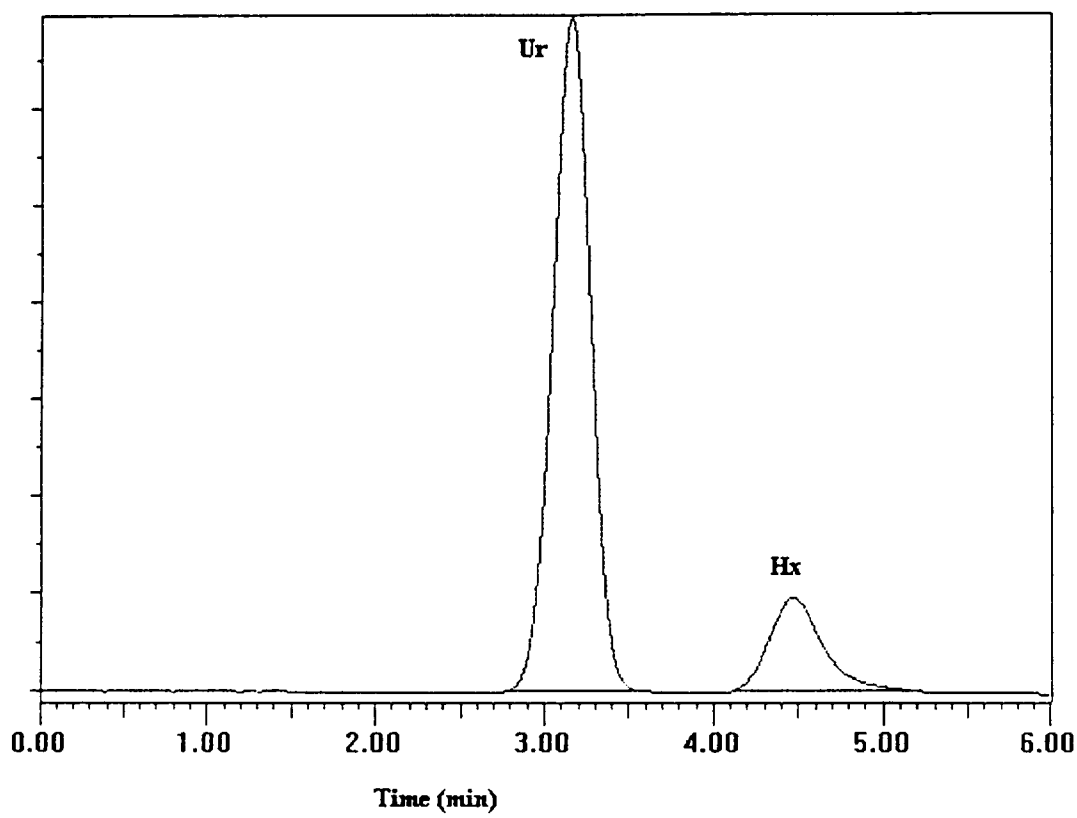


B)

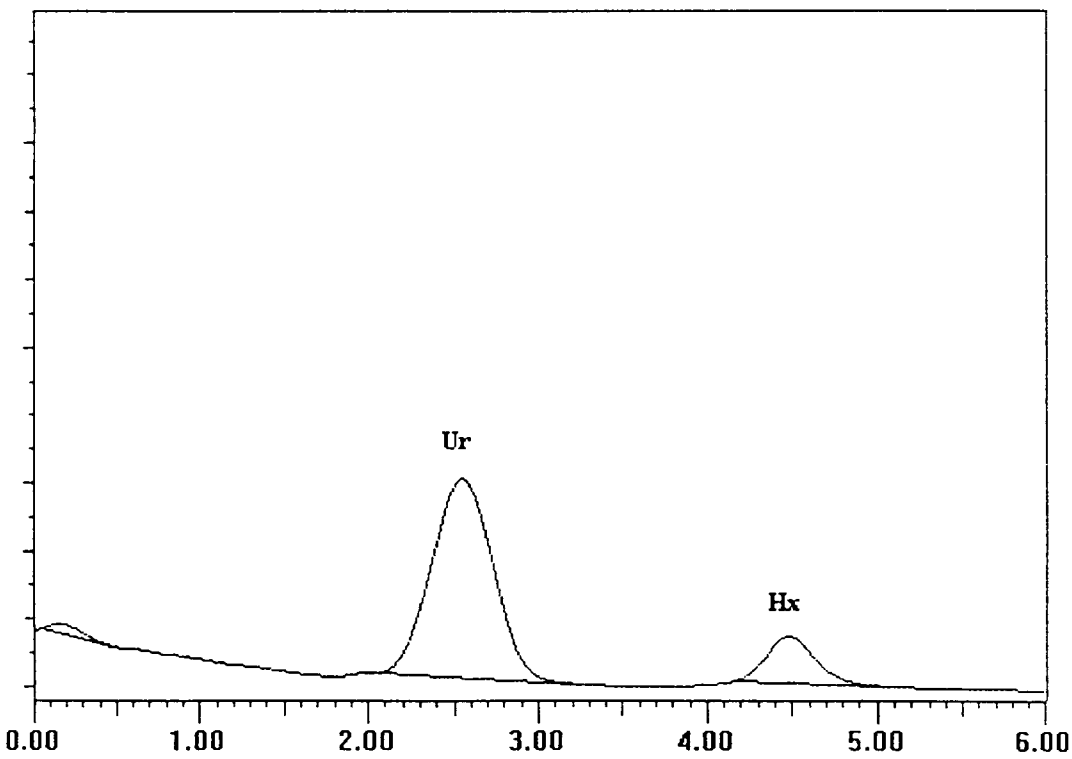


APPENDIX 4: EXAMPLE OF A CHROMATOGRAM FOR BOTH A) A STANDARD SOLUTION AND B) A BLOOD SAMPLE

A)



B)



APPENDIX 5: TABLE OF MEANS ± SEM FOR PLASMA NH_3, HX AND UR.

Plasma NH_3

Group	Placebo		Creatine	
Test	Test 1	Test 2	Test 1	Test 2
Time 0	50.6 (4.7)	33.9 (6)	47.3 (5.2)	39.6 (4.5)
10	71.2 (7.6)	81.5 (10)	97 (17.5)	82.4 (8.6)
20	95 (11.6)	100.1 (14.5)	128 (15.5)	102.3 (9.7)
40	99.3 (9.5)	114.2 (10.2)	131 (13.6)	102 (14.1)
60	98.4 (18.2)	135.5 (15.5)	145 (14)	105.9 (11.4)
62	98.4 (6.1)	111.8 (15.9)	132.1 (9.6)	106.6 (9.2)
66	84.3 (7.5)	94.6 (14.8)	113.3 (10.5)	91.8 (8.6)
70	79.2 (5.7)	85.5 (10.7)	87 (8.2)	69.2 (4.6)

Plasma Hx

Group	Placebo		Creatine	
Test	Test 1	Test 2	Test 1	Test 2
Time 0	1.6 (0.3)	2.3 (0.5)	2.7 (0.8)	2.2 (0.6)
20	3.4 (0.9)	5.1 (1.3)	10.3 (2.4)	6.2 (1.5)
40	3.2 (0.7)	3.1 (0.9)	10.6 (2.4)	6.3 (1.6)
60	3.3 (0.8)	6 (1.9)	11.7 (2.9)	5.2 (1.7)
62	4.7 (1.1)	8.7 (2.9)	15.6 (3.7)	6.8 (1.6)
66	7.5 (1.4)	12.1 (3.1)	16.7 (3.6)	11.2 (1.8)
70	9.2 (2.1)	10.1 (3.3)	15.9 (3.6)	12.1 (2.4)

Plasma Ur

Group	Placebo		Creatine	
Test	Test 1	Test 2	Test 1	Test 2
Time 0	314.9 (22.2)	303 (22.5)	318.9 (12)	271.8 (13.1)
20	318.1 (16.1)	305.8 (19.8)	339.9 (13.3)	288.5 15.2)
40	333.5 (21.2)	301.5 (17.4)	340.5 (16)	302.2 (14.4)
60	354.5 (15.3)	317.1 (20.2)	353.1 (15.2)	295.2 (13.5)
62	337.8 (14.2)	320.3 (19)	359.4 (12.3)	298.7 (13.6)
66	348.6 (22.5)	320.3 (17.8)	351.9 (15.5)	303 (13.7)
70	345 (22.7)	324.9 (24.4)	356.3 (14.2)	306.9 (16)