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**THE EFFECTS OF ORAL CREATINE
SUPPLEMENTATION ON HEALTH AND
DISEASE**



**UNIVERSITY
of
GLASGOW**

A thesis presented for the degree of Doctor of Philosophy
by

LIAM KILDUFF

*Centre for Exercise Science and Medicine
Faculty of Biomedical and Life Sciences
May, 2003*

"The history of science, like the history of all human ideas, is a history of irresponsible dreams, of obstinacy, and of error. But science is one of the very few human activities – perhaps the only one – in which errors are systematically criticised and fairly often, in time, corrected. This is why we can say that, in science, we often learn from our mistakes, and why we can speak clearly and sensibly about making progress there."

Sir Karl Raymund Popper, 1960.

DECLARATION

I hereby declare that this thesis has been composed by myself, that all the work of which is recorded has been done by myself except where assistance has been acknowledged, that it has not been submitted in any previous application for a higher degree and that all sources of information have been specifically acknowledged by means of reference.

Some of the results contained in this thesis have been published as follows:

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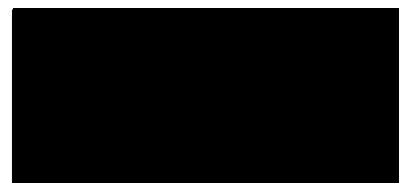
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L.P. Kilduff

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SUMMARY

The primary objectives of these experiments were to determine the effects of Creatine (Cr) supplementation on body composition and exercise performance in individuals of varying training and health status.

The aim of the first experiment was to determine the effects of Cr supplementation on muscle strength endurance and maximal performance in a group of resistance-trained males. This was achieved by comparing the effects of 20 g Cr·day⁻¹ for 5 days against the equivalent dose of a glucose polymer (placebo) in a group of 32 resistance trained individuals. The results of Experiment 1 demonstrated that Cr supplementation did not result in a significant increase in peak force or total work during repeated isometric contractions in resistance-trained individuals. However, this was due to the "non-responders" in the Cr group masking the effects of the remaining group ("responders"). When the Cr group was confined to only the "responders" to Cr, Cr supplementation resulted in significant increases in peak force and total force. This finding was further supported by the positive correlation between estimated muscle Cr uptake and increase in exercise performance, again suggesting that the efficacy of Cr supplementation is highly dependent on muscle Cr uptake. A negative correlation between training history and estimated muscle Cr uptake

was also found in Experiment 1, suggesting that training status may be a determinant of muscle Cr uptake potential.

The second experiment was designed to use strategies previously shown to optimise muscle Cr uptake and, hence, the potential to enhance exercise performance. The effects of 4 weeks of Cr supplementation allied with four weeks of resistance-training on isokinetic, isometric and isotonic strength were examined in previously non-resistance-trained humans. The results of Experiment 2 indicated that Cr supplementation in combination with strength training was effective in increasing isokinetic and isometric muscle strength but not 1 Repetition Maximum (1 RM) or training volume in subjects whose intramuscular [Cr] and body mass were significantly increased (i.e. responders). Furthermore, the greater the Cr uptake and associated increases in body mass, the greater were the exercise performance gains. Cr-stimulated increases in body mass and total body water compartments (TBW & ICW) were also observed.

The aim of the third experiment was to examine the effects of Cr-induced hyperhydration on cardiovascular, metabolic, and thermoregulatory responses to exercise, and on the capacity to perform prolonged exercise in the heat. The results of Experiment 3 suggest that Cr supplementation was effective in increasing predominantly intracellular water (ICW) and reducing cardiovascular

and thermoregulatory responses during prolonged exercise in the heat. The attenuation of these responses with Cr resulted in a significant increase in time to exhaustion (pre to post); this effect was only seen in subjects whose estimated intramuscular Cr levels were significantly increased following Cr supplementation (i.e., "responders" to Cr supplementation).

The aim of the fourth and final experiment was two-fold: firstly, to determine the effects of Cr loading on upper and lower body strength, upper and lower body strength endurance, and body composition; and secondly to examine the effects of Cr supplementation in conjunction with a standard pulmonary rehabilitation regimen on the above-mentioned variables in a group of patients with moderate to severe COPD. The results of Experiment 4 show positive results (lower body muscle strength, muscle endurance and upper body muscle endurance) with regard to the ergogenic potential for Cr in this patient group (both short-and long-term). However, it remains to be determined whether or not the performance benefits observed in the present study will have a positive impact on the patient's daily activities and, more importantly, their quality of life.

The results from all four experiments provide strong evidence that Cr supplementation is an effective strategy for:

- i) Increasing body mass in subjects ranging from highly trained athletes (Experiment 1 & 3) to patients with moderate to severe COPD (Experiment 4). However the exact mechanism behind these Cr stimulated increases remains to be determined.
- ii) Experiments 1 - 3 show Cr supplementation to be an effective ergogenic aid, only in subjects whose estimated muscle Cr uptake was significantly elevated following supplementation. The failure of many studies to characterise the Cr group on this basis could help explain the reasons behind the conflicting results with regard to the ergogenic effect of Cr supplementation.
- iii) And finally, the results of Experiment 4 point towards a possible role for Cr supplementation in the rehabilitation process for patients with COPD, with this study being the first to show improvements in muscle strength and endurance following intervention in this patient group.

TABLE OF ABBREVIATIONS

ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BIA	Bioelectrical impedance analyser
BM	Body mass
BMI	Body mass index
CK	Creatine kinase
CNS	Central Nervous System
Cr	Creatine
Crea	Creatinine
CHO	Carbohydrate
CO ₂	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CV	Coefficient of variation
Db	Body density
ECW	Extracellular water
FFM	Fat-free mass
FFMI	Fat-free mass index
FM	Fat-mass
H ⁺	Hydrogen

TABLE OF ABBREVIATIONS (cont.)

Hb	Haemoglobin
Hct	Hematocrit
HR	Heart rate
5-HT	5-hydroxytryptamine
ICC	Intra-class correlation
ICW	Intracellular water
La ⁻	Lactate
MVC	Maximal voluntary contraction
Na ⁺	Sodium ions
O ₂	Oxygen
PCr	Phosphocreatine
PCV	Packed cell volume
Pi	Inorganic phosphate
³¹ P-MRS	³¹ Phosphorus nuclear magnetic resonance imaging
RER	Respiratory exchange ratio
RM	Repetition maximum
RPE	Rating of perceived exertion
RPM	Revolutions per minute
rhGH	recumbent human Growth Hormone

TABLE OF ABBREVIATIONS (cont.)

s.d.	Standard deviation
TBW	Total body water
T_{skin}	Weighted mean skin temperature
[TCr]	Total creatine concentration
T_{rec}	Rectal temperature
Vb	Body volume
$\dot{V}O_{2 \text{ max}}$	Maximal oxygen uptake
$\dot{V}CO_2$	Volume of carbon dioxide produced
WR_{max}	Maximum work rate
Δ	Delta

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CHAPTER ONE

General Introduction

GENERAL INTRODUCTION

Introduction

Fatigue is an inevitable consequence of sustained physical activity, with fatigue commonly defined as an inability of muscle to maintain the required force or power output (Edwards, 1981). The physiological, psychological and biochemical processes that result in fatigue have been an area of extensive research for many years. Although still far from fully understood, the mechanisms behind exercise fatigue have become clearer in recent years. The underlying mechanisms vary according to the mode, duration and intensity of exercise, training, nutritional and motivational status of the subject, and also with environmental conditions in which the exercise is carried out. In recent years, researchers have attempted to characterise the fatigue process under all the above-mentioned conditions. A widely studied fatigue protocol is that of exercise performed to the limit of tolerance at an exercise intensity of 50–60% of maximum oxygen uptake ($\dot{V}O_{2\max}$) under thermo neutral conditions. Mechanisms that have been associated with fatigue during this type of exercise include depletion of intramuscular glycogen stores and hypoglycaemia e.g. Coyle *et al* (1985). At this exercise intensity, muscle glycogen is the primary substrate utilized (Bergstrom & Hultman, 1967), and although exercise can

continue once glycogen stores become depleted, it can only be at a reduced intensity, and the criterion of fatigue is thus met.

At the other end of the exercise spectrum is short-duration high intensity exercise. The causes of fatigue during this type of exercise have also been extensively investigated, yet are still not fully characterised. Fatigue during this type of exercise was initially attributed predominantly to factors such as intracellular accumulation of inorganic phosphate (Pi) and/or hydrogen ions (H⁺) (e.g. Maughan *et al*, 1997). However, Katz *et al* (1986) and, more recently, Hultman *et al* (1990) demonstrated that fatigue during short-term, high intensity exercise was associated with a low intramuscular phosphocreatine concentration ([PCr]), rather than a high lactate concentration ([La⁻]). This led both groups of researchers to suggest that substrate availability (PCr), as opposed to product inhibition, might be the important determinant of fatigue during high-intensity short duration exercise. Furthermore Hultman *et al* (1990) demonstrated that PCr depletion coincided with the decline in isometric force during a single bout of intermittent electrical stimulation. As a result, the decline in ATP production during this type of exercise has been attributed to a reduced capacity to resynthesize ATP, due to the depletion of PCr stores (Soderlund *et al*, 1991).

Exercise physiologists have examined strategies for delaying the fatigue process in order to further understand the mechanistic basis of fatigue. In recent years,

for example, researchers have attempted to delay fatigue through the use of nutritional ergogenic aids. It has been well established that carbohydrate supplementation prior to and during exercise can delay the onset of fatigue and thus increase performance during prolonged sub-maximal exercise (Coyle *et al*, 1985; Coggan & Coyle, 1989) but, until recently, there has been little systematic investigation of the effects dietary supplementation may have on the performance of high-intensity exercise. However, over the last decade researchers have begun to examine the potential effects of creatine (Cr) monohydrate supplementation on the fatigue process during high-intensity short duration exercise.

Historical Background

Creatine was first identified in 1835 by a French scientist called Chevreul (Dermant & Rhodes, 1999). However, it was not until 1847 that Lieberg confirmed its presence as a regular constituent of flesh extracted from mammals (Needham, 1971). Following this, Lieberg also reported that the flesh of wild foxes killed in the chase contained 10 times as much Cr as that of captive creatures, therefore concluding that Cr accumulation was associated with muscular exercise (Dermant & Rhodes, 1999). At around the same time, Heintz and Pettenkofer discovered a substance in the urine which Lieberg later confirmed as being creatinine (Crea), which is now known to be a by-product of

Cr degradation. However it was not until the early twentieth century that researchers started to examine the effects of Cr ingestion on muscle [Cr]. Around this time, it was also discovered that not all the ingested Cr was retrieved in the urine, leading researchers to conclude that the body retained some of the ingested Cr (Dermant & Rhodes, 1999). The first documented studies to confirm this were by Denis (1912) and Folin (1914) on the muscle of cats. These researchers both reported that, following Cr ingestion, muscle [Cr] increased by ~70%. In 1927 and 1929, Fiske and Subbarow reported the presence of labile phosphorus in resting cat muscle, which they subsequently called creatine phosphate or phosphocreatine (PCr). The same researchers also showed that, during electrical stimulation of skeletal muscle, [PCr] decreased for a period of time, then returning to resting levels following a rest period. The results of these several studies, taken together, thus lead to the subsequent identification of free intramuscular Cr (Cr_{free}) and PCr, and also highlighted their potential role in skeletal muscle metabolism (for discussion see Balsom *et al*, 1995). However, it is only within the last decade or so that systematic research into the effects of Cr supplementation on muscle metabolism and performance has been undertaken.

Biochemistry of Creatine

Creatine (Cr), or methylguanidine-acetic acid, is a naturally occurring compound synthesized from three amino acids: arginine, glycine, and

methionine. This process (transamidination) starts with the transfer of an amidine group from arginine to glycine, which leads to the formation of guanidinoacetate and ornithine, and is a reversible reaction catalysed by the enzyme glycine-amidine-transamidinase. Cr is then formed by the addition of a methyl group from (S)-adenosylmethionine (transmethylation), which requires the enzyme methytransferase for the irreversible reaction (Figure 1.1) (Devlin, 1992). The enzymes involved in the synthesis of Cr are located in the kidney, liver and pancreas. However, as the main site of Cr utilisation is skeletal muscle (95% of the body's total creatine concentration is found in skeletal muscle) (Walker *et al*, 1979), Cr must be transported from its site of synthesis to its site of utilisation (skeletal muscle) via the blood stream (Figure 1.2). Having arrived at its site of utilisation, uptake of Cr occurs against a concentration gradient. Cr enters muscle cells via Na⁺-dependent sarcolemmal transporters (Zorzano *et al*, 2000). The endogenous production and dietary intake of Cr is matched by the degradation of PCr and Cr to creatinine (Crea) at a rate of 2.6% and 1.1% per day, respectively (Wyss and Kaddurah-Daouk, 2000). Once Crea is formed, it enters the circulation by diffusion and is eliminated from the body through glomerular filtration (Figure 1.2).

Figure 1.1: **Synthesis of Creatine (Wyss and Kaddurah-Daouk, 2000)**

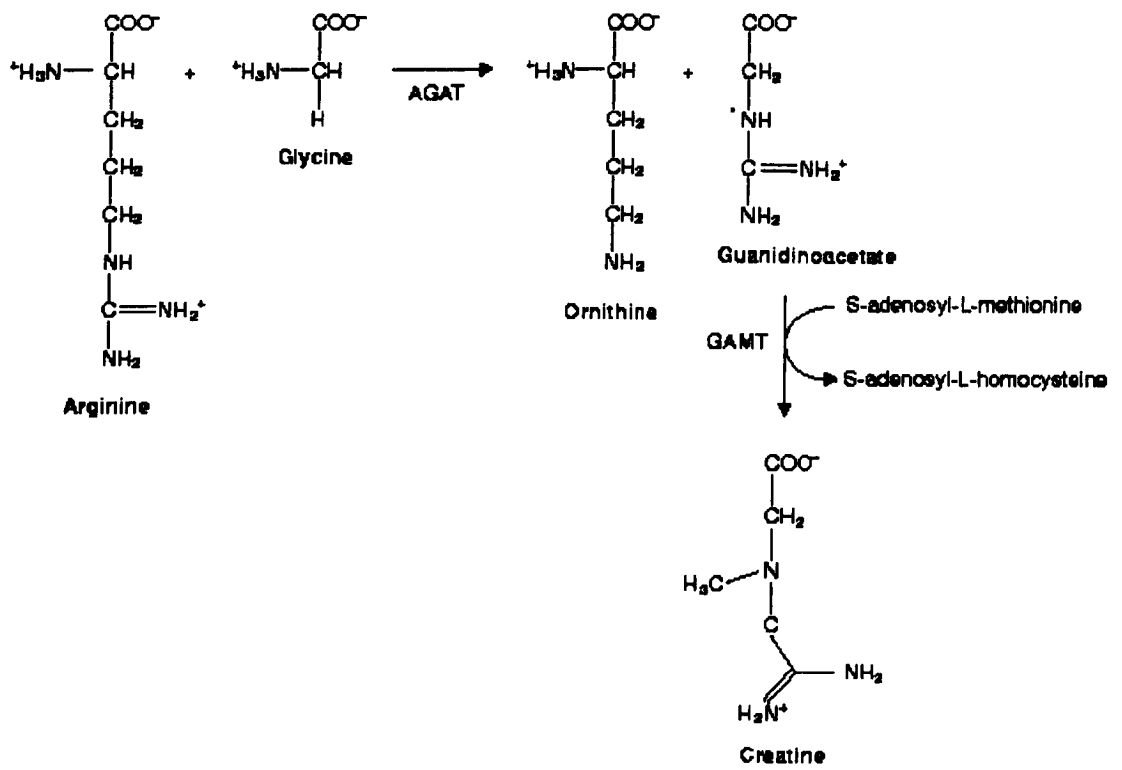
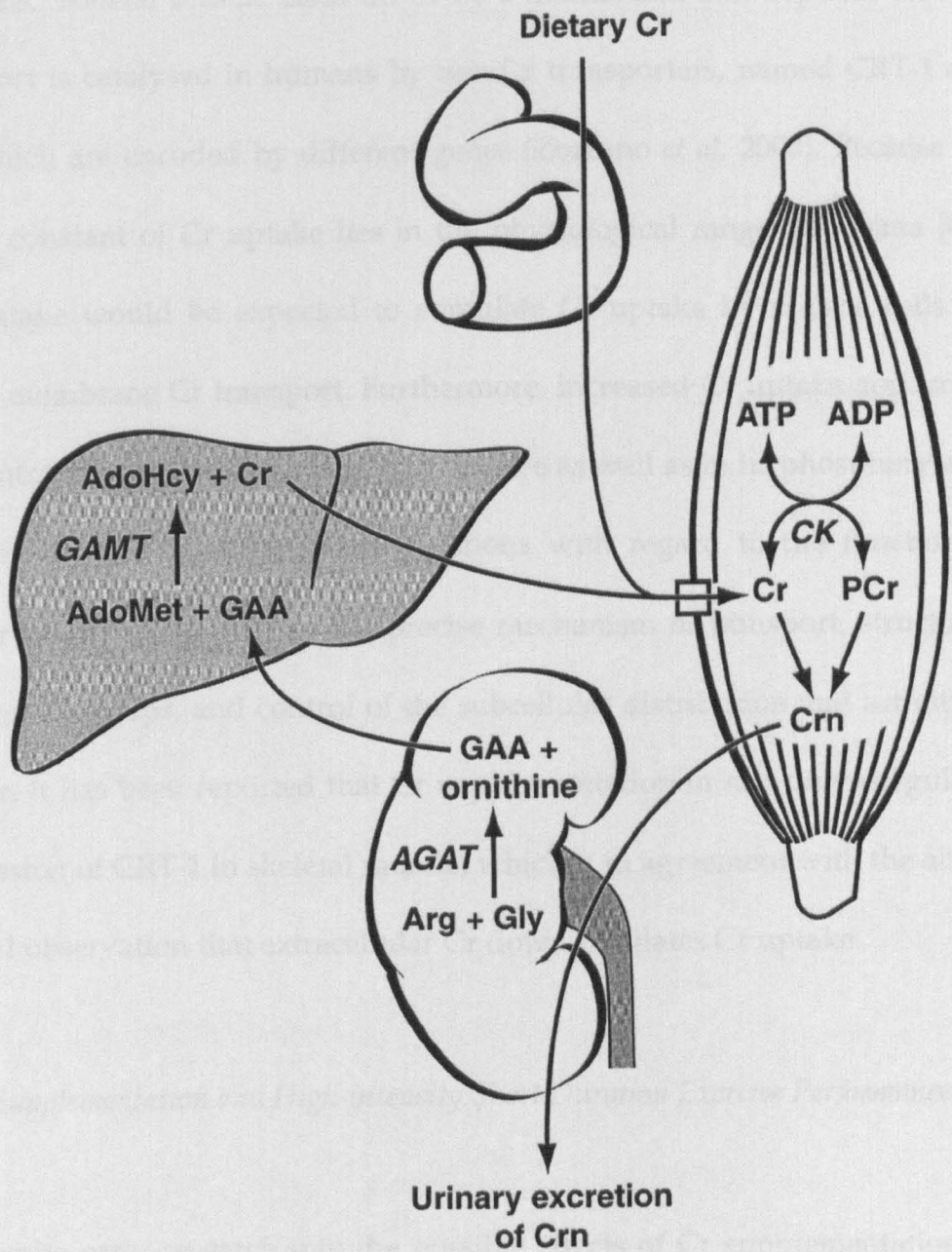


Figure 1.2:

Overview of Creatine Supplementation (Wyss and Kaddurah-Daouk, 2000)



Creatine Transporters

Specific Cr transporters present in the sarcolemma facilitate Cr entry into muscle cells. Skeletal muscle takes up Cr by a mechanism that depends on Na⁺. Cr transport is catalysed in humans by two Cr transporters, named CRT-1 and CRT-2, which are encoded by different genes (Zorzano *et al*, 2000). Because the Michaelis constant of Cr uptake lies in the physiological range of plasma [Cr], oral Cr intake would be expected to stimulate Cr uptake by muscle cells via enhanced membrane Cr transport. Furthermore, increased Cr uptake appears to result in intracellular Cr accumulation, in its free as well as in its phosphorylated form. There are many unanswered questions with regard to the function of muscle Cr transporters, such as the precise mechanism of transport, structure/function relationships, and control of the subcellular distribution and activity of the carrier. It has been reported that Cr supplementation in rats down-regulates the expression of CRT-1 in skeletal muscle, which is in agreement with the above mentioned observation that extracellular Cr down-regulates Cr uptake.

Creatine Supplementation and High-intensity Short Duration Exercise Performance

Despite early research into the possible effects of Cr supplementation on muscle metabolism, it was not until 1992 that researchers began to reinvestigate the possible effects of Cr loading on human skeletal muscle metabolism (Harris *et*

al, 1992). Harris *et al* (1992) were the first to investigate whether Cr supplementation could increase [TCr] of human skeletal muscle, and whether such an increase in plasma [Cr] might ultimately lead to an increase in total Cr content ([TCr]) of skeletal muscle, as had previously been suggested. Thus Harris *et al* (1992) supplemented a group of subjects with 5g Cr for 4-6 times a day for 3 or more days. Muscle biopsies were taken from the vastus lateralis pre and post supplementation. Although the supplementation dosage and duration were not standardised, this study provided important information with regard to Cr supplementation and muscle Cr uptake (Harris *et al*, 1992). Firstly, muscle biopsy analysis showed that following Cr supplementation the [TCr] increased from 126.8 to 148.6 mmol/kg dry mass, with the increases in PCr accounting for 20-40% of the total increase. Secondly, the increase in muscle [TCr] was reported to vary significantly among subjects. This was attributed to subject heterogeneity with regard to Cr uptake, i.e. some subjects being "responders" and others "non-responders". However, the difference in dosage regimens might also account for some of this variability. In addition, subjects with the lowest initial [TCr] showed the greatest Cr uptake following supplementation. Finally, the greatest intramuscular uptake of Cr was reported to occur over the initial days of supplementation (urinary analysis showed 40, 61 and 68% of the supplemented dose was recovered on days one, two and three, respectively), with the authors suggesting there might be a limit to the amount of Cr that can be stored within muscle.

This seminal work by Harris *et al* (1992) was the catalyst for the subsequent plethora of studies that have examined the effects of Cr supplementation on muscle metabolism and performance. Many researchers have confirmed the increases in muscle [TCr] following Cr supplementation. For example, Greenhaff *et al* (1994) observed increases of 15 -32% (29 ± 3 mmol/kg dry mass) in [TCr] following 5 days of Cr supplementation. Other studies that have also found similar increases in [TCr] and PCr following Cr supplementation using various techniques ranging from muscle biopsy analysis to ³¹P-phosphorus nuclear magnetic resonance imaging (³¹P-MRS) (Balsom *et al*, 1995; Casey *et al*, 1996; Green *et al*, 1996; Hultman *et al*, 1996; Smith *et al*, 1998; Vandenberghe *et al*, 1997).

The effects of Cr supplementation on performance have also been examined. Greenhaff *et al* (1993) were the first to investigate the potential role of Cr supplementation as an ergogenic aid. They examined 12 physically active, but not highly trained young male subjects. Following familiarisation, knee extensor performance was measured on an isokinetic dynamometer before and after 5 days of Cr supplementation (20 g d⁻¹). Subjects performed 5 sets of 30 maximal voluntary contractions (unilateral knee extensions) with 1 minute between bouts. This study demonstrated that the 5 days of Cr supplementation increased total peak torque during the 2nd and 3rd bout of contractions, with a strong tendency in the 4th set. Peak torque generation was also significantly increased during the final 10 contractions in the 1st bout and during contractions 11 - 20 in the 5th bout.

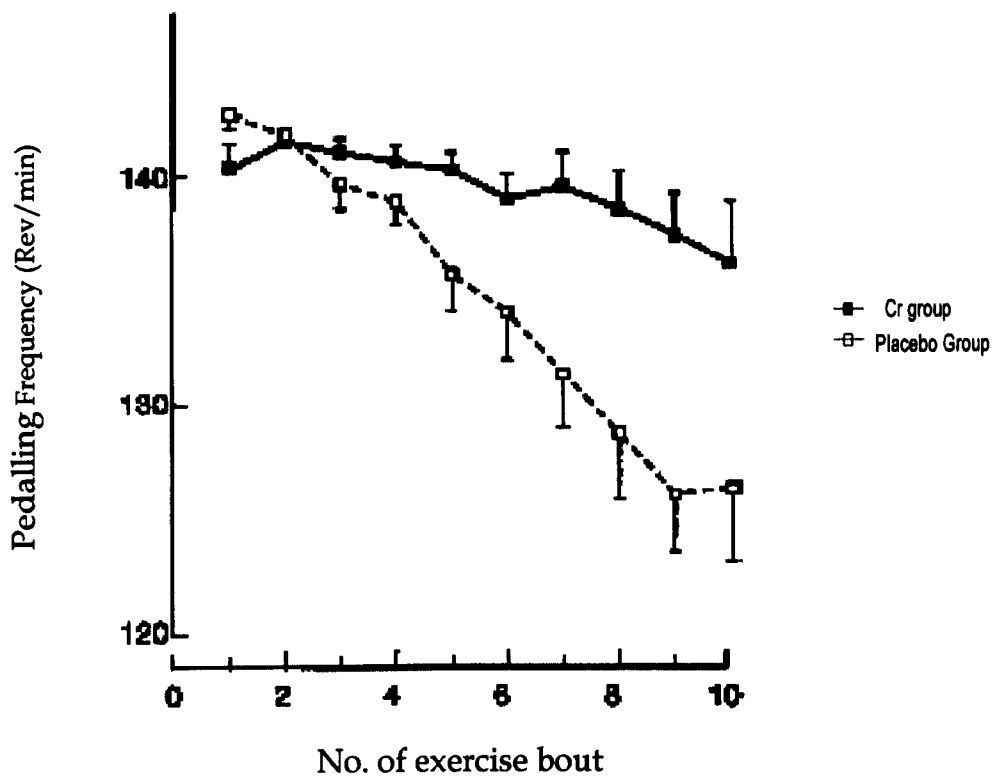
Consistent with these results, significantly lower plasma [ammonia] (an accepted marker of muscle adenine nucleotide loss) after the 4th and 5th bouts was reported.

In the same year, Balsom *et al* (1993) confirmed the results of Greenhaff *et al* (1993). In this study, subjects performed an exercise protocol that consisted of ten 6-s bouts of high-intensity cycle-ergometer exercise, with 30 s recovery between repetitions (subjects being instructed to attempt to maintain a pedalling frequency of 140 rpm). This exercise protocol was carried out pre and post 6 days of Cr loading at a dosage of 20 g·d⁻¹. Subjects were found to better maintain the target pedal frequency following Cr supplementation, compared to the placebo group (Figure 1.3).

Many researchers have subsequently continued to examine the effectiveness of Cr supplementation as an ergogenic aid. For example other reported benefits resulting from Cr loading include increases in maximal voluntary contraction (MVC) and muscle endurance capacity (Maganaris & Maughan, 1998), improved anaerobic sprint performance during repeated bouts of exercise (Casey *et al*, 1996; Green *et al*, 1996, Balsom *et al*, 1993; Earnest *et al*, 1995), and increased performance during single exhausting bouts of high intensity exercise (Earnest *et al*, 1995; Rossiter *et al*, 1996). However, not all studies have reported Cr to have

Figure 1.3:

Effect of Cr supplementation on muscle performance during high-intensity cycling (Balsom *et al.* 1993)



an ergogenic effect. For example, several studies found no ergogenic benefit of Cr loading on either intermittent (Odland *et al*, 1997; Febbraio *et al*, 1995; Gilliam *et al*, 2000) or continuous (Redondo *et al*, 1996; Burke *et al*, 1996; Cooke *et al*, 1995; Deutekom *et al*, 2000) exercise tests.

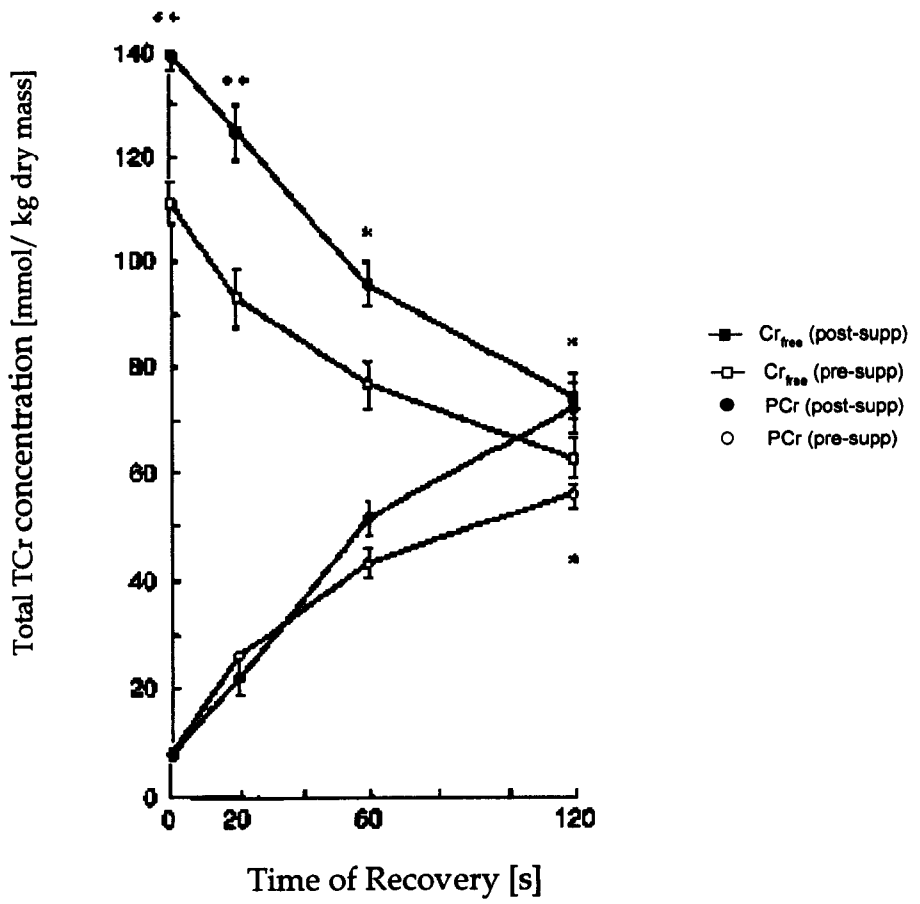
The mechanism of action by which Cr supplementation improves performance is still not fully understood. However, the two most commonly proposed to date are a higher pre-exercise intramuscular [PCr] and/or a higher rate of PCr resynthesis between bouts of exercise (Greenhaff *et al*, 1994). The energy required to perform brief explosive-type exercise is almost exclusively provided by the high-energy phosphate stores in skeletal muscle (Fitch *et al*, 1974). As the PCr stores become depleted, performance rapidly deteriorates which reflects the inability to rephosphorylate ADP to ATP at the required rate (Hultman *et al*, 1990). The increased intramuscular PCr store post-supplementation is proposed to act as a temporal energy buffer which, in turn, would decrease reliance on anaerobic glycolysis. Increases in the resting [PCr] may allow subjects to complete more work during short-duration high intensity exercise (Casey *et al*, 1996). Secondly, many authors (Harris *et al*, 1992; Greenhaff *et al*, 1993; Soderlund *et al*, 1994; Balsom *et al*, 1993; Balsom *et al*, 1995) have proposed that Cr supplementation would increase the rate of PCr resynthesis from mitochondrial ATP, during recovery, consequent to the elevated muscle Cr content (Harris *et al*,

1992) increasing the rate of flux through the creatine kinase reaction at the mitochondrial membrane.

Greenhaff *et al* (1994) followed up their initial study (Greenhaff *et al*, 1993) to more directly investigate the effects of Cr supplementation on PCr resynthesis in skeletal muscle. In this study, the authors electrically stimulated the anterolateral portion of the thigh (vastus lateralis) in 8 male subjects. Following the electrical stimulation, a pressure cuff was inflated around the thigh (259 mmHg) to occlude blood flow and a muscle biopsy then obtained. The cuff was then deflated and further muscle biopsies were obtained at 20, 60 and 120 s. Subjects were supplemented with 20 g·d⁻¹ of Cr for 5 days, and then returned to the laboratory to repeat exercise protocol. The results of this study showed that Cr supplementation increased the rate of PCr resynthesis by 42% during the second minute of recovery (Figure 1.4). In addition, only those subjects who evidenced a substantial increase in resting muscle [TCr] following Cr supplementation showed an increased rate of PCr resynthesis during recovery; those whose muscle [TCr] increased only marginally (<10 mmol·kg⁻¹·dry weight muscle) showed very little change in PCr resynthesis.

Since this work by Greenhaff and co-workers (1994), several other groups of researchers have investigated the effects of Cr supplementation on PCr resynthesis but with conflicting results. For example, Yquel *et al*, (2002)

Figure 1.4: Phosphocreatine and free creatine concentrations obtained after 0, 20, 60 and 120 s of recovery from intense contractions before (open symbols) and after (closed symbols) Cr ingestion (Greenhaff *et al.* 1994). * and ** indicates difference between pre and post-supplementation ($P < 0.05$ and $P < 0.01$, respectively).



examined the effects of 6 days of Cr supplementation on muscle power, muscle PCr resynthesis, Pi and pH during repeated bouts of maximal dynamic plantar flexion exercise. The authors used ^{31}P -MRS to assess the changes in skeletal muscle high-energy phosphate status during five bouts of 8 s interspersed with 30 s recovery, followed by 6 bouts of 8 s and 7 bouts of 16 s separated by 1 and 2 min, respectively. Subjects were tested pre and post 6 days of Cr supplementation. Following supplementation, muscle power increased by 5% from bouts 3 to 7 and muscle PCr resynthesis increased during the 10 min recovery period. These results are in agreement with the earlier results obtained by Greenhaff *et al* (1994), with the authors concluding that the observed increases in muscle power were the result of a lower accumulation of inorganic phosphate and a less-acid intramuscular pH during exercise, and a higher rate of PCr resynthesis in recovery. Smith *et al* (1998) also verified the results obtained by Greenhaff *et al* (1994), using ^{31}P -MRS. They showed muscle PCr resynthesis was increased following Cr supplementation in middle-aged and younger persons after subjects performed single-leg knee extension dynamic exercise.

However, not all studies have reported a more rapid increase in PCr resynthesis in recovery from intense exercise following Cr supplementation (Vandenberghe *et al*, 1999; Francaux *et al*, 2000). Vandenberghe *et al* (1999), used ^{31}P -MRS to examine the effects of Cr supplementation on muscle PCr breakdown and resynthesis and muscle performance during high-intensity intermittent knee

extensions. Interestingly, these authors found a significant increase in performance following Cr supplementation, despite Cr supplementation not affecting PCr breakdown or resynthesis during and after isometric muscle contractions, respectively.

Creatine and Training

In parallel with the investigations described above, several groups have explored the influence of Cr supplementation on the outcomes of exercise training programmes. The physiological basis underlying a possible ergogenic effect of Cr supplementation on strength training is primarily two-fold. Firstly, Cr supplementation has been shown to increase, for example, the numbers of repetitions that can be completed per set (Earnest *et al*, 1995; Volek *et al*, 1997). Secondly, Cr supplementation has been shown to increase the rate of PCr resynthesis during the second minute of recovery from intense intermittent type exercise (Greenhaff *et al*, 1994). Theoretically, both these physiological changes could allow an individual to train at a greater intensity compared to training without the use of Cr supplementation.

Becque *et al* (2000) examined the effects of 6 wk of Cr supplementation combined with resistance training (elbow flexors training twice a week with training loads that began at 6 Repetition maximum (RM), the maximum weight that can be

lifted 6 times and progressed to 2 RM). In this study, Cr supplementation during elbow flexor training lead to significantly greater increases in 1 RM elbow flexor strength, upper arm muscle area (7.9 cm²), and fat-free mass (FFM) compared to training alone. Vandenberghe *et al* (1997) (10 wk resistance training programme, where subjects trained their upper and lower body 3 hr·wk⁻¹), also reported positive effects of Cr supplementation when combined with resistance training, compared to resistance training alone. In this study, maximal strength of the trained muscle groups, maximal intermittent exercise capacity of the elbow flexors, and FFM increased 20 - 25%, 10 - 15% and 60% more, respectively, than the group that trained without Cr supplementation. Cr supplementation combined with resistance training (varying duration 4-12 wk.) has also been shown to increase bench press lifting volume (Kreider *et al*, 1998), total sum of bench press, squat and power clean lifting volume (Kreider *et al*, 1998), total work performed during five 6-s sprints (Kreider *et al*, 1998), 1 RM squat and bench press (Volek *et al*, 1999), average lifting volume in the bench press (Volek *et al*, 1999), muscle strength (Willoughby & Rosene, 2001). Additionally, all the above studies reported significantly greater gains in FFM following Cr supplementation when combined with resistance training compared to resistance training alone (Kreider *et al*, 1998; Volek *et al*, 1999; Willoughby & Rosene, 2001).

However, not all studies have reported positive effects on muscle strength following combined Cr supplementation and resistance training. Francaux and

Poortmans (1999) examined the effects of 6 weeks of resistance training in conjunction with Cr supplementation on isokinetic squat force and found that Cr ingestion did not induce a greater increase in force compared to resistance training alone; isokinetic force increased by about 6 % after training in both the placebo and Cr groups. However, there are several concerns with this study: there was no measure or estimate of Cr uptake; and questions must be asked about the training "stimulus" (i.e. only 30 % of MVC in session 1, increasing progressively to approximately 43 % of MVC in the final training session). Similarly, Bermon *et al* (1998) examined the effects of Cr supplementation in conjunction with 7 weeks of resistance training on strength and strength endurance in 32 elderly subjects. They also found that Cr supplementation did not provide any additional benefit to maximal dynamic strength compared to resistance training alone.

Creatine and Endurance Exercise

In contrast, there has been little systematic study of the effects of Cr supplementation on endurance performance. Although the majority of research to-date has examined the role of PCr as a temporal energy buffer and its effects on short-term high intensity exercise, it has recently been proposed that [PCr] may play a pivotal role in the control of muscular oxygen consumption and

pulmonary oxygen uptake ($\dot{V}O_2$) (Whipp & Mahler, 1980, Barstow *et al*, 1994; McCreary *et al*, 1996; Rossiter *et al*, 1999).

In recent years, a number of researchers have investigated the dynamic profiles of $\dot{V}O_2$ and [PCr] in humans, in order to investigate the control of muscle O_2 consumption. Barstow *et al* (1994) and McCreary *et al* (1996) were two of the first groups of investigators to examine this in humans, following on from the work by Mahler (1985) in frog muscle. However, as pointed out by Rossiter *et al* (1999), the above-mentioned studies were not without methodological constraints. For example, in the paper by Barstow *et al* (1994), despite reporting similar time constants for [PCr] depletion and increase in $\dot{V}O_2$ with square-wave exercise, the authors utilised different muscle groups operating over different metabolic ranges to examine the time constants for [PCr] and $\dot{V}O_2$. Therefore Rossiter *et al* (1999) furthered the initial work by Barstow *et al* (1994) by simultaneously measuring the kinetics of [PCr] and $\dot{V}O_2$ during moderate intensity exercise of the *m. quadriceps* muscle in a NMR magnet. The time constant for intramuscular [PCr] depletion (35 s, range, 20 - 64 s) was almost identical to the time constant for the $\dot{V}O_2$ kinetics (phase II, only) (36 s, range, 20 - 68 s). This study further supported the possible role of [PCr] in the control of $\dot{V}O_2$. As previously mentioned, the ergogenic potential of Cr supplementation is based on the premise that, following Cr supplementation, resting levels of [PCr] are elevated.

Therefore, based on the work of Rossiter *et al* (1999), the observed increase in resting [PCr] following Cr supplementation might have the potential also to alter the kinetics of $\dot{V}O_2$ and hence of muscle O_2 consumption. To date, only one study has examined this possibility (Jones *et al*, 2002). Jones *et al* (2002) examined the effects of 5 days of Cr loading (20 g d⁻¹) on $\dot{V}O_2$ during moderate and heavy exercise. Cr loading caused a significant reduction in $\dot{V}O_2$ during heavy exercise but not moderate exercise; a finding that warrants further investigation.

Other studies have also examined the effects of Cr supplementation on muscle bioenergetics during incremental and submaximal exercise tests with negative results. For example, Stroud *et al* (1994) and Balsom *et al* (1993) reported no significant effect on respiratory gas exchange ($\dot{V}O_2$, $\dot{V}CO_2$, RER) variables following Cr supplementation, with Stroud *et al* (1994) also reporting no effect on blood [lactate].

Exercise in the Heat and Creatine Supplementation

While the majority of the available research suggests that Cr supplementation has no ergogenic effect on endurance exercise, recently some researchers have begun to investigate the potential role for Cr supplementation during exercise in the heat due to the already mentioned Cr stimulated changes

in body composition. To date, 3 studies have examined the effect of Cr supplementation on performance during exercise in the heat (Vogel *et al*, 2000; Volek *et al*, 2001; Kern *et al*, 2001). Volek *et al* (2001) examined the effect of Cr supplementation on acute cardiovascular, renal, temperature and fluid-regulatory hormonal responses during 35 min of exercise in the heat (37 °C, 80 % relative humidity), which was immediately followed by three 10-s sprints. In this study, the authors found a significant increase in body mass (0.75 kg) following Cr supplementation, which led to a significantly greater peak power during all three 10 s sprints, compared to no change in the placebo group. No significant change in heart rate, blood pressure, and sweat rate responses was observed following Cr supplementation, indicating that it is unlikely that Cr significantly influenced temperature regulation during 35 min of exercise in a hot humid environment. Furthermore, no abnormal responses in several measures of renal function were found during rest and exercise.

More recently Kern *et al* (2001) examined the effects of 28 days of Cr supplementation on heart rate and core temperature during 60 min of exercise at an intensity equal to 60% $\dot{V}O_2$ max at 37 °C and 25 % relative humidity. They observed significantly greater gains in body mass and Total Body Water (TBW) compared to the placebo group, with a consequent attenuation of core temperature during post-supplementation. The increases in body mass and TBW observed in the above study and the attenuation in rectal temperature were of

similar magnitude as those observed in the present study. However, in the study by Kern *et al* (2001), subjects exercised for a fixed period of time (60 min) and therefore we are unable to evaluate if the decrements in core temperature had any effect on performance during exercise in heat.

Enhancing Muscle Creatine Uptake

As already mentioned, one of the preliminary findings from Harris *et al.* (1992) was that the ergogenic and metabolic effects of Cr supplementation seem to be dependent on the magnitude of the associated increase in muscle TCr. More specifically, Greenhaff *et al* (1994) and Casey *et al* (1996) suggested that an increase in muscle [TCr] in excess of 20 mmol·kg⁻¹·dry weight muscle is required to elicit an ergogenic effect on muscle power output and post-exercise PCr resynthesis. Casey *et al* (1996) also observed that changes in both maximum work production and total work production following Cr supplementation were correlated with muscle Cr uptake. As a result of this positive correlation between Cr uptake and increase in performance observed by Casey *et al* (1996) and Greenhaff *et al* (1994), researchers have explored strategies that might have the potential to facilitate muscle Cr uptake during Cr supplementation, thus leading to greater gains in performance.

To date, two main strategies for enhancing muscle Cr uptake during supplementation have emerged. Firstly, the early work of Harris *et al* (1992) was the first study to examine whether Cr uptake by skeletal muscle could be increased by exercise. Thus, Harris *et al* (1992) employed 1 hour of one-legged cycling exercise per day for 4 - 7 days and demonstrated enhanced Cr uptake in the exercising leg by 54 %, whilst little effect was observed in the non-exercising leg. The precise mechanism(s) for the exercise-induced increase in muscle Cr uptake is still unknown, although an increase in blood flow to the exercising muscle and/or changes in the sarcolemmal transport kinetics of Cr were suggested by the authors as the most plausible explanations. Subsequently, however, Robinson *et al* (1999) suggested that the results obtained by Harris *et al* (1992) should be treated with caution, citing: the relatively small number of subjects studies, the variation in the supplementation regimens, and the inclusion of vegetarians. Robinson *et al* (1999) therefore studied 14 subjects who performed one-legged cycling exercise to exhaustion with muscle biopsies (vastus lateralis) taken from the exercised and non-exercised leg immediately after exercise, 6 hr and 5 days post-recovery. They showed that a greater muscle [TCr] was achieved in the exercised limb, but disputed the hypothesis of Harris *et al* (1992) that increased muscle blood flow was a possible cause for the increased TCr in the exercised limb. This was made on the basis that no differences were observed at the 6 hr muscle biopsy between the exercised and

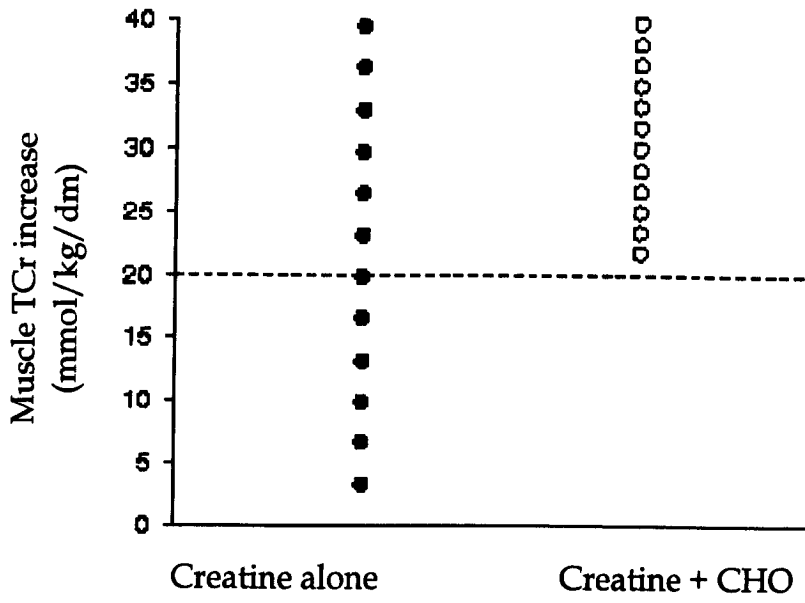
non-exercised limbs when differences in blood flow to the limbs would be at their greatest.

An additional strategy shown to enhance muscle Cr uptake is the ingestion of carbohydrate in combination with Cr. Insulin, at supra-physiological concentrations, has previously been shown to increase muscle Cr uptake in rat skeletal muscle cells in vivo (Haughland and Chang, 1975). More recently, supra-physiological [insulin] has also been shown to stimulate Cr uptake in a mouse myoblast cell line (Odoom *et al*, 1996). Subsequently many researchers began to examine the effects carbohydrate supplementation may have on muscle Cr uptake in humans. In 1996, Green *et al* (1996) examined this in human subjects: group A, consumed Cr alone; group B consumed Cr combined with a carbohydrate-containing solution; group C consumed Cr combined with a carbohydrate-containing solution and also performed 1 hr of cycling exercise at 70% of their $\dot{V}O_{2\max}$ on the morning of each day; and group D consumed a solution free from Cr and carbohydrate. Evidence for an enhanced muscle Cr uptake associated with carbohydrate came from a markedly reduced peak plasma [Cr] and area under the plasma Cr/time curve in subjects consuming carbohydrate-containing solution in combination with Cr supplementation (groups B & C), compared with subjects who consumed Cr alone (group A). Urinary Cr excretion was also shown to be lower in groups B and C, compared to that of group A. Green *et al* (1996) thus concluded that (a) whole-body Cr

retention was increased as a consequence of Cr being combined with carbohydrate (Figure 1.5) and (b) the response occurred as a consequence of an insulin-mediated increase in skeletal muscle Cr uptake. In addition, as insulin has also been shown to increase muscle blood flow (Baron *et al*, 1994), the carbohydrate-stimulated increase in muscle Cr uptake in humans could result in part from an insulin-mediated increase in muscle blood flow and thereby muscle Cr availability. However, in the study by Green *et al* (1996), subjects had to ingest 94g of carbohydrate (in the form of simple sugars) to achieve physiologically high plasma [insulin] during the 1st hour after ingestion, which proved to be close to the limit of subject palatability and this study was also unable to identify the exact [insulin] necessary to stimulate muscle Cr uptake. It was for this reason, Steenge *et al* (1998) went on to try and identify the [insulin] necessary to stimulate muscle Cr uptake. Steenge *et al* (1998) infused insulin at rates of 5, 30, 55, or 105 mU.m⁻².min⁻¹ in combination with 12.4 g.d⁻¹ of Cr. This study confirmed the findings of Green *et al* (1996) showing that, during infusion rates of insulin at 55 and 105 mU.m⁻².min⁻¹, muscle [Cr] increased by 4.5±1.4 and 8.3±1.0 mmol.kg⁻¹.dry weight muscle respectively, and plasma [Cr] was lower at specific time points compared with the lower infusion rates. This study demonstrated that insulin can enhance muscle [Cr] uptake in humans but only when at physiologically high or even supra-physiological concentrations. The authors also suggested that the mechanism behind this response was likely to be

Figure 1.5:

Individual muscle total Creatine [TCr] after ingestion of 5g Cr alone or 5g Cr followed 30 mins later with 93g simple carbohydrate (Green *et al.* 1996)



a result of an insulin-mediated increase in muscle Cr transport rather than an increased rate of vascular Cr delivery.

Creatine and health

As with any relatively new ergogenic aid, especially one suggested to be as potent as Cr supplementation, questions concerning health effects have arisen. To date, the only documented side effect resulting from Cr supplementation has been a significant weight gain (Earnest *et al*, 1995; Green *et al*, 1996; Greenhaff *et al*, 1994; Maganaris & Maughan, 1998; Terrillion *et al*, 1997; Becque *et al*, 2000; Kreider *et al*, 1998; Harris *et al*, 1992; Poortmans and Francaux, 1999). However, there have been numerous anecdotal reports reporting gastrointestinal, cardiovascular and muscular problems following Cr supplementation. Therefore, researchers have examined the effects of Cr supplementation of various health markers over short -, medium-, and long-term supplementation.

For example, Robinson *et al* (2000) examined the effects of Cr supplementation (20 g·d⁻¹ of Cr for 5 days, followed by 3 g·d⁻¹ of Cr for nine weeks) on various markers of muscle damage, and indices of haematological, hepatic and renal function. No clinically significant changes were observed in any of these variables. Another study examining the effects of short to medium Cr supplementation on blood lipids was carried out by Volek *et al* (2000). These

authors examined the effects of Cr supplementation in combination with heavy resistance training on fasting serum creatinine, lipoproteins, triglycerides and reported changes in body function. Again, following Cr supplementation and training for 12 weeks, there were no adverse effects of training or supplementation on fasting serum creatinine, serum total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides or reported changes in body function.

In contrast, Earnest *et al* (1996) showed Cr supplementation to have a positive effect on blood lipid profile. This study showed that 8 weeks of Cr in conjunction with an exercise programme consisting of resistance training actually increased HDL- cholesterol by 13%.

Waldron *et al* (2002) also examined the effects of concurrent Cr supplementation and resistance training on markers of hepatic function. Subjects were loaded for 5 days ($0.3 \text{ g.kg}^{-1}.\text{d}^{-1}$ of Cr) followed by 5 weeks of maintenance ($0.03 \text{ g.kg}^{-1}.\text{d}^{-1}$ of Cr) during which subjects weight-trained. Hepatic stress was assessed through measurement of serum concentrations of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (AP), blood urea nitrogen (BUN), albumin, creatinine, and direct and total bilirubin (DB and TB). This study concluded that, following Cr loading and maintenance,

concentrations of the measured variables did not change, with all variables remaining within normal clinical ranges.

Finally, Mihic *et al* (2000) examined the short- to medium-term effects of Cr supplementation on health variables. In this study, the authors examined the effects of acute Cr loading (20 g·d⁻¹ of Cr for 5 days) on blood pressure, plasma creatinine and plasma creatine kinase in a group of 30 men (n=15) and women (n=15). Cr supplementation was found to have no negative effects on blood pressure, plasma creatinine, estimated creatinine clearance or plasma creatine kinase activity.

However, some studies have raised questions over the long term safety of Cr supplementation. For example, Pritchard and Kalra (1998) recently proposed that oral Cr supplementation may lead to renal dysfunction. Poortmans and Francaux (1999) and Schilling *et al* (2001) therefore subsequently examined the effects of long-term Cr supplementation on various health markers. For example, Poortmans and Francaux (1999) measured creatinine, urea and plasma albumin clearances in consumers of Cr supplementation of durations varying from 10 months to 5 years against a control group. The results of this study showed there were no statistical differences between the control group and the Cr group for plasma contents and excretion rates of creatinine, urea and albumin. Clearance of these compounds also did not differ between the two groups, implying a lack of

effect on glomerular filtration rate, tubular reabsorption and glomerular membrane permeability. Subsequent to this, Schilling *et al* (2001) also examined the long-term safety of Cr supplementation. In this retrospective study, the authors examined markers of health, incidence of reported side effects and perceived training benefits in athletes supplementing with Cr for 0.8 to 4 years. The authors subjected the athletes to a standard clinical examination including 27 blood chemistries; subjects also answered a questionnaire on dietary habits, Cr supplementation, medical history, training history and perceived benefits of supplementation. The data from this study again suggested that long-term Cr supplementation did not result in any adverse side effects.

Creatine and body composition

As already mentioned, the only consistently reported side effect with regard to Cr supplementation (short- and long-term) is an increase in body mass (Earnest *et al*, 1995; Green *et al*, 1996; Greenhaff *et al*, 1994; Maganaris & Maughan, 1998; Terrillion *et al*, 1997; Becque *et al*, 2000; Kreider *et al*, 1998). Although the exact mechanism behind this Cr-related increase in body mass is unknown, two possible mechanisms have been proposed.

The first possible cause stems from the early work of Ingwall *et al* (1972 & 1974). The first of these studies (Ingwall *et al*, 1972) presented evidence that Cr

supplementation was involved in the control of muscle protein synthesis. In 1974, Ingwall *et al* (1974) followed up their initial study and found that increasing the amount of Cr availability to differentiating skeletal muscle cells both in vitro (in monolayer culture) and in vivo (explants maintained in organ cultures) stimulated the rate of myosin heavy chain synthesis two-fold.

Further support for this hypothesis came from the work by Sipila *et al* (1981). This experiment was designed to examine the effects of Cr supplementation as a treatment for gyrate atrophy of the choroid and retina. In this condition, marked progressive atrophy of the Type II skeletal muscle fibres is consistently reported, with the authors stating that deficient formation of Cr may be a pathogenic component of this disease which may lead to a shortage of cellular PCr energy stores. Seven patients were supplemented with 1.5g creatine daily for one year. On completion of the study, Type II muscle fibres increased from 34.1 ± 7.1 to 49.9 ± 7.0 μM , with the authors suggesting that these changes were a direct result of a Cr stimulated increase in protein synthesis.

The other possible mechanism is that the increase in body mass following Cr supplementation is due to an increase in water retention (Hultman *et al*, 1996). This theory stems from the work of Hultman *et al* (1996). In this study, these authors observed a 0.6 L decline in urinary volume which occurred at the onset of Cr ingestion, and suggested that the increase in body mass during acute Cr

loading is likely to be attributed to body water retention. The authors also noted that the time course of the urinary volume changes paralleled the time course of muscle Cr uptake documented by Harris *et al* (1992).

The exact mechanisms by which Cr supplementation increases total body water (TBW) and shifts fluid into the intracellular space still remain unclear. One possible cause is that osmotic flux of fluid into the intracellular compartment is caused by the increased [TCr] and [Cr] within the muscle, thus increasing intracellular water (ICW) and hence TBW, ultimately increasing muscle volume.

Ziegenfuss *et al* (1998) examined the effects of Cr supplementation on acute fluid volume changes following 3 days of Cr supplementation. In this study the researchers supplemented their subjects with Cr ($0.35 \text{ g kg} \cdot \text{FFM}^{-1} \cdot \text{d}^{-1}$) for 3 days, and found Cr supplementation produced clear trends in fluid shifts, and by the end of day 3 increased TBW (2%) and ICW (3%), with no change in Extracellular water (ECW).

In view of all the above, the main objectives of this series of experiments were to investigate:

- i. In light of limitations in the previous research (e.g. small sample size and inappropriate statistical analysis) the first experiment in this thesis was designed to examine the effects of Cr supplementation on strength endurance and maximal performance using an isometric bench-press test in a group of resistance-trained males.
- ii. Based on the results obtained from the first experiment, a second was designed to use strategies previously shown to optimise muscle Cr uptake and, hence, the potential to enhance exercise performance in previously non-resistance-trained humans on isokinetic, isometric and isotonic strength.
- iii. The results from Experiment 2 indicated a Cr-induced change in cellular hydration. Exercise in the heat has been shown to be critically dependent on hydration status and the aim of the third experiment was, therefore, to examine the effects of Cr-induced hyperhydration on cardiovascular, metabolic and thermoregulatory responses to exercise in the heat. Furthermore, the influence of Cr-induced hyperhydration on the subject's capacity to perform prolonged exercise in the heat was also examined.

iv. Skeletal muscle dysfunction is frequently observed in Chronic Obstructive Pulmonary Disease (COPD) patients. These patients suffer from significant loss of skeletal muscle mass and strength (reduced muscle mass is an important predictor of mortality). As Cr supplementation has the potential to increase muscle strength, muscle endurance and fat-free mass (FFM), the aim of the final study was to examine the effects of Cr supplementation in conjunction with a standard pulmonary rehabilitation on patients with moderate to severe COPD. The effects of Cr loading on upper and lower body strength, upper and lower body strength endurance and body composition were studied.

CHAPTER TWO

General Methods

GENERAL METHODS

This chapter describes the general methodology used throughout this thesis. This thesis comprises of four main experimental chapters (chapters 3 - 6). Methods specific to each of these chapters can be found in the relevant experimental chapters (chapters 3 - 6). Only methods common to all experiments will be discussed here i.e., dietary analysis, urinary analysis, body composition.

Subjects and Study Approval

All experiments described in this thesis involved human volunteers. The subject groups used in this thesis were as follows: highly resistance-trained males (Experiment 1, chapter 3), non-resistance trained males (Experiment 2, chapter 4), endurance trained male cyclists (Experiment 3, chapter 5) and patients with moderate to severe chronic obstructive pulmonary disease (COPD) (Experiment 4, chapter 6). Inclusion criteria for each subject group can be found in the individual experiment methods sections.

Experiments 1-3 were approved by the University of Glasgow Ethics Committee; Experiment 4 was approved by the Glasgow Royal Infirmary Ethics board. The nature and purpose of each experiment was explained both verbally and in writing to each subject / patient prior to each experiment. Subjects were also

made aware that they could withdraw from the studies at any time without explanation. All subjects provided written informed consent prior to taking part in the experiment (Appendix 1).

Experimental Design

All four experiments followed a double-blind placebo controlled design. A significant limitation in this design is the lack of a crossover, i.e. subjects acting as their own controls. However, the use of a crossover design is problematic in the design of Cr supplementation studies. This is primarily due to the slow washout kinetics of Cr (4 - 8 wks) from muscle, making it difficult to interpret results obtained from the placebo trial when administered as the second treatment, as can be seen from the study performed by Maganaris & Maughan (1998). Therefore for this reason a double-blind placebo controlled design was carried out with subjects in each group matched for body mass or performance (see individual experiment chapters for specific details). Much care was taken to ensure there was no order effect on performance by including a minimum of two familiarisations trials before subjects entered the experiment phase of each study, details of specific familiarisations are given in each Experimental chapter.

Familiarisation and Repeatability of Tests

All subjects underwent at least two familiarisation tests except for Experiment 4 where due to the subject group (patients with moderate to severe COPD) used only one familiarisation trial was possible. During all familiarisation trials, subjects were taken through the exact experimental procedure and related protocols in order to fully familiarise the subjects with the experimental conditions and related exercise protocols (see individual Experimental chapters for specific criteria).

Dietary Analysis

Energy intake and diet composition were determined for each subject following completion of a weighed intake of varying duration, which was dependent on the specific study (see Experimental Chapters for details). During the weighed intake, subjects were asked to follow their normal diet (except for the extra carbohydrate contained in the experiment drinks) and weigh and record all food and drink consumed over the required period. Weighing scales accurate to ± 1 g were given to all subjects. The weighed dietary intake was used to determine energy intake and dietary composition using a computerised version of McCance & Widdowson's food composition tables as revised by Holland *et al* (1991). These results were used to ensure any observed changes in

body composition and/or performance were not the result of changes in dietary intake.

Urinary Analysis

Urinary [Crea] and [Cr] in studies 1 - 3 were determined for each subject following 24 hour urinary collection of varying duration, which was dependent on the specific study (see Experimental Chapters for details). All urine was collected over a 24 hour period in a 5 L container provided by the investigators, subjects began urinary collection the day before supplementation in all experiments in order to obtain baseline results. The volume of urine collected for each 24 hour period was measured and mixed thoroughly, with two representative 20 ml samples being stored at -20° C for subsequent analysis of [Cr] and [Crea] using a spectrophotometric enzymatic Crea Kit (Boehringer Mannheim MPR1 - Kit no. 839434) on a ABX Mira Plus Spectrophotometer (ABX Diagnostics, UK). The Crea content of the urine was measured by a sequence of four enzymatic steps as shown below:

- 1st step: Creatinine + H₂O creatininase Cr
- 2nd step: Cr + H₂O creatinase Sarcosine + Urea
- 3rd step: Sarcosine + H₂O + O₂ sarcosine oxidase Glycine + HCHO + H₂O₂
- 4th step: H₂O₂ + Phenol derivative + 4-aminophenazone peroxidase red benzoquinone imine dye

Following the measurement of [Crea], the [Cr] content of the urine was then measured which involved the removal of the first step of the above creatininase reaction, with the remaining three enzymatic reactions (2nd to 4th step) being completed as described by Oversteegen *et al* (1987). Total Cr excretion was corrected for any observed increase in Crea following supplementation. Estimated Cr uptake was calculated by subtracting the total Cr excreted, corrected for [Crea] excretion, from the total amount supplemented per day. Estimated intramuscular [Cr] (mmol·kg⁻¹·dry weight muscle) was calculated based on an estimated muscle mass amounting to 40% of body mass and average muscle water content approximating 77% of wet weight (Bergstrom *et al*, 1971) as previously described by Maganaris & Maughan (1998). The method used to estimate Cr uptake in the present set of Experiments has previously been used as the sole method to estimate Cr uptake (e.g. Rossiter *et al*, 1996, Maganaris & Maughan, 1998) and in conjunction with the measurement of Cr uptake obtained from muscle biopsies (Harris *et al*, 1992, Hultman *et al*, 1996, Green *et al*, 1996). However this method is based on two major assumptions, firstly, 40% of body mass is muscle mass and secondly, average muscle water content is approximately 77% of wet weight. Utilising the 40% of body weight estimate of muscle mass is almost certainly an underestimation in some of our subject groups (Experiments 1-3). We therefore also analyzed the data using estimated muscle mass of $\pm 5\%$ (i.e. 35 and 40%) to estimate Cr uptake (for this we utilised the data obtained from Experiment 1). Utilising these two additional estimates of

muscle mass (35 and 45%) altered the absolute Cr uptake values; however, the 4 subjects who were "non-responders" remained "non-responders" and the 17 subjects remained "responders". In addition, we also recognise that the fixed muscle hydration level of 77% may also affect the results obtained especially due to the fact the Cr supplementation has been shown to increase cellular hydration levels. We therefore reanalyzed the data using estimated muscle hydration levels of $\pm \sim 30\%$ (47 and 100%) to estimate Cr uptake, again for this we utilised the data obtained from Experiment 1. Utilising these two additional estimates of muscle hydration (47 and 100%) altered the Cr uptake values dramatically; however, the 4 subjects who were "non-responders" remained "non-responders" and the 17 subjects remained "responders". We have therefore opted to use the previously published and referenced 40% of body mass is muscle mass and average muscle water content is approximately 77% of wet weight.

Body Composition

Due to the reported changes in body composition following Cr supplementation, body composition analysis was completed in all four studies.

Body Mass

Body mass was measured, wearing underwear/ swimsuit only, using a calibrated Seca electronic scale with precision of ± 0.01 kg (Seca, Isle of Man). The subjects/ patients stood on the platform, facing away from the scales, with their body weight evenly distributed between both feet.

Body Composition Measurements

Body composition was measured using a bioelectrical impedance analyser (BIA) (Bodystat-1500, Bodystat Ltd, Douglas, UK in Experiments 1 & 4 and Bodystat-5000, Bodystat Ltd, Douglas, UK in Experiments 2 & 3). This procedure allows measurement of fat mass (FM), fat-free mass (FFM), total body water (TBW) using the Bodystat-1500 impedance analyser and body water compartments (intracellular (ICW) and extracellular (ECW) water), using the Bodystat-5000 impedance analyser.

Body composition measurement by bioelectrical impedance was performed on the right side, with subjects/ patients supine, and with their limbs slightly apart from the trunk. Two current-introducing electrodes were placed on the dorsal surfaces of the right hand and foot proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively. Then two detector electrodes were

placed on the right pisiform prominence of the wrist, the proximal edge dissecting the ulnar tubercle, and between the medial and lateral malleoli, the proximal edge dissecting the medial malleolus. The impedance to current flow between the injector and detector electrodes was determined. These placements are standard placements for electrodes during measurement using BIA (van Loan, 1990). Preceding the electrode placement, the skin had been cleaned with 70% alcohol to remove dead skin and dirt before electrode placement. BIA measures the opposition of body tissues to the flow of small (less than 1mA) alternating current. Impedance is a function of two components (vectors): the resistance of the tissues themselves, and the additional opposition (reactance) due to the capacitance of membranes, tissue interfaces, and non-ionic tissues. The measured resistance is approximately equivalent to that of muscle tissue.

Throughout all experiments the National Institute of Health (NIH) standardisation procedures were followed (NIH, 1994) which were as follows:

All measurements were performed on a non-conductive surface in an environmental chamber ($\sim 23^{\circ}\text{C}$) with the arms and legs slightly abducted ($\sim 30^{\circ}$) from the trunk. Subjects were also required to abstain from alcohol, caffeine, and heavy exercise for 24 hrs prior to testing. Finally, subjects were given a 10 min supine equilibration period before each measurement.

BIA has recently become a popular method of estimating body composition due to it being a safe, convenient, portable method and its ability to perform frequent, rapid and non-invasive measurements (Koulmann *et al.* 2000). In recent years, many researchers have attempted to assess the validity and reliability of this method of estimating body composition. While there is still much debate with regard to the validity (limits of agreement) of BIA estimation of body composition, the majority of researchers have concluded that BIA is a repeatable method (Kushner & Schoellor, 1986; Baumgartner *et al.*, 1990; Koulmann *et al.*, 2000; Fornetti *et al.*, 1999). Koulmann *et al.* (2000) reported the mean reproducibility for trial to trial intra-individual impedance measurements at 5 kHz was 4.4% (coefficient of variation (CV)) and 2.2 % (CV) at 100 kHz, with these variations being slightly greater than those reported by Kushner & Schoeller (1986) (2.2 % week to week CV).

In a separate investigation we assessed the short-term reliability (7 days) of BIA on 25 healthy male subjects for the measurement of FFM, TBW and ICW using Bland & Altman (1986) limits of agreement. In this study we found the mean bias and 95% confidence intervals to be -0.1 ± 0.9 kg, -0.2 ± 0.9 L and -0.1 ± 0.6 L for FFM, TBW and ICW, respectively. Further support for the short-term reliability of BIA can be seen in all four Experimental chapters when comparing the pre-post values obtained from the placebo groups. The non-significant change in any

of the BIA determined body composition variables obtained from the placebo group in Experiments 1 - 4 is further support for the reliability of BIA.

Experimental Controls

Subjects were also requested to eliminate caffeine and caffeine-containing foods from their diet over the loading period in all 4 experiments to minimize the possible inhibitory effects of caffeine on the ergogenic effect of Cr (Vandenberghe *et al*, 1996). In Experiments 1 and 3, subjects were required to maintain their normal training habits for the duration of the study. Experiments 2 and 4, subjects underwent supervised training and it was stressed that all other activity was to be kept within their normal habits. At the end of the all experiments, subjects gave verbal and written assurance that they had complied with these instructions.

Cr Dosage and Carbohydrate

All four experiments involved Cr and placebo supplementation. Due to variations in the experimental design and experimental needs, Cr dosages varied between experiments. Also due to the proposed stimulatory effect of carbohydrate on muscular Cr uptake (Green *et al*, 1996), subjects also consumed extra carbohydrate with their Cr dosages. In Experiment 1, subjects consumed

90g of glucose polymer in combination with Cr (as recommended by Green *et al*, 1996); however this proved to be at the subject's limit of tolerance. Therefore, in subsequent experiments (2 - 4), subject's consumed a moderate amount of carbohydrate with their Cr dosage (35g per 5g Cr), and were also instructed to consume the Cr following meal times (additional carbohydrate). The exact Cr dosages and supplementation periods are stated in the methods section of each Experimental chapter.

Data Analysis

Due to the similar experimental design of all studies (placebo controlled) data analysis was similar for all experiments.

Data was expressed as the mean \pm s.d. or median (range), following a test for the normality of distribution. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by Student's paired *t*-test (within treatment effect, i.e., pre- vs. post-supplementation) and two-sample *t*-test (between treatment effect, i.e., magnitude of change in the Cr group or "responders" vs. the placebo group) if a main treatment or interaction effect was observed. An ANCOVA was used where necessary to normalise for differences in pre-supplementation results using the baseline value as the covariate.

Pearson's correlation analysis was used to assess the relationship between selected variables. Statistical significance was declared at $P \leq 0.05$.

CHAPTER THREE

(Experiment 1)

Effects of Creatine Supplementation on Isometric Bench- Press Performance in a Group of resistance-trained Humans

INTRODUCTION

The energy required to perform brief explosive-type exercise is almost exclusively provided by the high-energy phosphate stores in skeletal muscle. As the phosphocreatine (PCr) stores become depleted, performance rapidly deteriorates, reflecting the inability to rephosphorylate adenosine diphosphate (ADP) to adenosine triphosphate (ATP) at the required rate (Hultman *et al*, 1990). Increasing resting levels of intramuscular creatine (Cr) by oral Cr supplementation (Harris *et al*, 1992) has been shown to increase intramuscular PCr levels and to accelerate the resynthesis of ATP during and following high-intensity, short-duration exercise (Balsom *et al*, 1995; Greenhaff *et al*, 1994).

In recent years, numerous studies have investigated the effects of Cr supplementation on exercise performance and body composition, but with conflicting results. A significant limitation in the design of Cr supplementation studies is that the use of a crossover design is problematic. That is, the slow washout kinetics of Cr from muscle makes it difficult to interpret results obtained from placebo trials when administered as the second treatment. This methodological problem has forced many investigators to use matched subject groups. Many such studies have also used too few subjects (i.e. < 20), thus increasing the chance of producing a Type II error (Tarnopolsky & MacLennan, 2000). The risk of producing a Type II error is also increased if subjects

supplemented with Cr are not differentiated into "responders" and "non-responders" on the basis of measured or estimated Cr uptake (Casey *et al*, 1996; Greenhaff *et al*, 1994). Another problem inherent in using matched subject groups is the choice of appropriate statistical methods. For example, some of the positive findings in the literature (e.g. Birch *et al*, 1994 and Harris *et al*, 1992) have been questioned because of the use of multiple t-tests instead of analysis of variance (ANOVA), therefore increasing the probability of committing a Type I error (Gilliam *et al*, 2000).

With the above concerns in mind, the balance of available evidence from Cr supplementation studies would suggest that oral Cr loading can increase muscle Cr content (Balsom *et al*, 1995; Harris *et al*, 1992; Hultman *et al*, 1996), increase fat-free mass (Becque *et al*, 2000; Kreider *et al*, 1998), improve anaerobic sprint performance (Casey *et al*, 1996; Green *et al*, 1996) and promote greater gains in strength (Kreider *et al*, 1998; Maganaris & Maughan, 1998; Volek *et al*, 1997). Although the effects of Cr supplementation on exercise performance have been investigated in a number of different subject groups using a variety of different intervention strategies and exercise modes, little systematic investigation has been given to the effects of Cr supplementation on performance of isometric exercise. The aim of the present Experiment was therefore to determine the effects of Cr supplementation on strength endurance and maximal performance using an isometric bench-press test in a group of resistance-trained males.

Although the isometric bench-press test is unable to replicate the typical bench-press manoeuvre (i.e. the test is isometric, while the training or competitive manoeuvre is isotonic), it partly simulates the training adopted by resistance-trained subjects, and also allows force to be measured with a high degree of accuracy.

METHODS

Subjects

Thirty-two healthy resistance-trained males (Table 3.1), from whom written informed consent had been obtained, volunteered to take part in this Experiment which was approved by the local ethics committee. Subject eligibility was initially assessed by interview. No subject had a history of cardiovascular or respiratory disease and/or evidence of musculoskeletal injury. Subjects were recruited on the basis that they were engaged in a structured weight-training program at the time of recruitment. All subjects had at least 2 years training experience and were demonstrated not to have supplemented with Cr for at least 8 weeks prior to the Experiment. Investigators did not reveal prior to interview that subjects would be excluded if they had supplemented with Cr in the last 8 weeks. Eight subjects (four in each group) had previously supplemented with Cr. No Cr was detected in the baseline urine samples of any subject. Subjects typically undertook 3 - 4 resistive-training sessions per week, with an emphasis on major muscle groups. All subjects completed at least one heavy dynamic bench-press session per week (e.g. two warm-up sets at ~50% of the subject's one repetition maximum (1 RM), pyramiding up to 1 RM within 4 sets).

Table 1: Physical characteristics of the two groups of subjects

	Placebo Group (n=11)		Creatine Group (n=21)	
	Pre	Post	Pre	Post
Age (yr)	24 ± 5	-	24 ± 5	-
Height (m)	1.79 ± 0.08	-	1.79 ± 0.07	-
Weight (kg)	80.1 ± 7.3	80.2 ± 7.1	84.1 ± 8.2	85.1 ± 8.0
Total Body Water (l)	48.4 ± 3.7	48.5 ± 3.9	49.9 ± 4.1	50.4 ± 4.2
Total Body Water (%)	60.0 ± 3.6	60.6 ± 2.8	59.5 ± 3.0	59.4 ± 3.0
Fat-free mass (kg)	69.3 ± 5.6	69.4 ± 5.8	71.9 ± 5.7	72.6 ± 5.7
Fat-free mass (%)	6.7 ± 4.1	86.7 ± 4.1	85.7 ± 3.5	85.5 ± 3.4
Body Fat (kg)	10.9 ± 3.8	10.8 ± 3.7	12.2 ± 3.9	12.6 ± 3.8
Body Fat (%)	13.4 ± 4.1	13.3 ± 4.1	14.3 ± 3.5	14.5 ± 3.4
Peak power (N)	815 ± 255	-	812 ± 207	-
Training history (yr)	5 ± 2	-	5 ± 2	-

Values are presented as the mean ± s.d.

Experimental Design

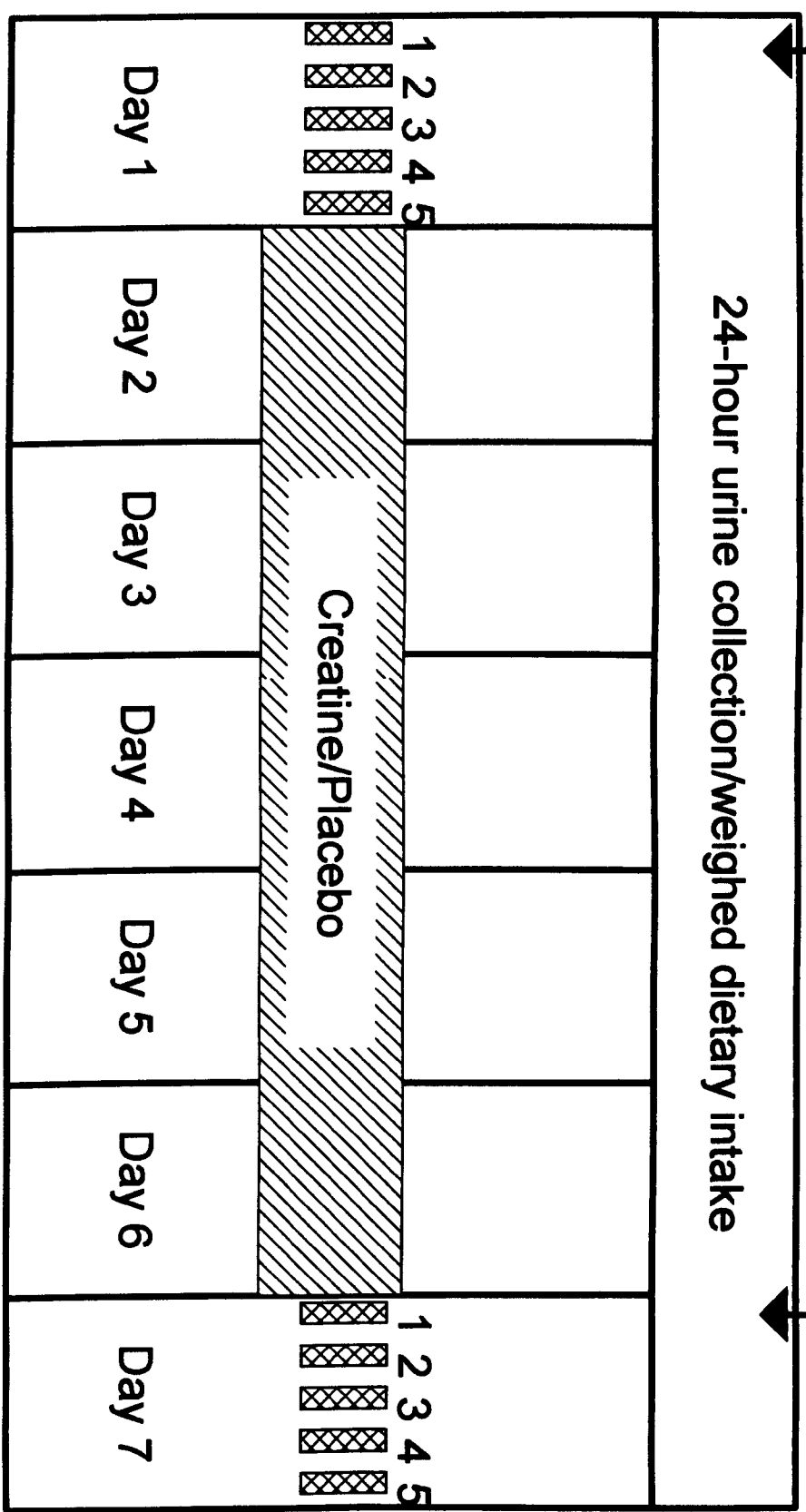
Prior to entering the experimental phase of the Experiment, subjects visited the laboratory on at least two occasions in order to become familiar with the isometric bench-press and related protocols. Familiarisation trials were carried out until the variability of two consecutive performances was within 100 N for peak force. The test-retest reliability of the isometric bench-press revealed a high intra-class correlation (ICC) for both performance outcomes (Peak force ICC = 0.95, Total force ICC = 0.95; these test-retest reliability values are based on subjects having each undergone three familiarization tests). On the basis of the final familiarization results (peak force), subjects were assigned in a double-blind fashion to either a Cr group or a placebo group on a two to one ratio; this asymmetry was designed to accommodate for both "responders" and "non-responders" to Cr supplementation (Greenhaff *et al*, 1994). Following the familiarization period, all subjects performed two isometric bench-press tests at least five days apart. The first isometric bench-press test was conducted 48 hours after the subject's final familiarization trial. The supplementation period for both groups started on the day after the first isometric bench-press test and finished the day before the second isometric bench-press test. The experimental design is shown in Fig. 3.1.


Figure 3.1

The experimental design for Experiment 1

Body weight
Total body water

Body weight
Total body water



 20-sec maximal isometric bench-
press exercise

The Cr group ingested $22.8 \text{ g} \cdot \text{d}^{-1}$ Cr·H₂O (equivalent to 10 g Cr × 2 daily) for 5 days before and after each daily training session. Each pre- and post-work out supplement dose consisted of 11.4 g of Cr·H₂O (equivalent to 10 g Cr) and 90 g of glucose polymer made up in 500 mls of warm to hot water. This regimen was adopted in light of the work by Harris *et al* (1992) who found that this protocol increased resting muscle PCr levels within 5 days. Rossiter *et al* (1996) also used a 5 day Cr supplementation period in trained individuals and found ergogenic effects. Dissolving Cr in warm to hot water prevented any detectable formation of creatinine (Crea), with no parts of the supplement remaining undissolved. The addition of dextrose to the Cr has been shown to significantly enhance the uptake of Cr (Green *et al*, 1996; Steenge *et al*, 1998). On training days, subjects consumed the first Cr dose 1 h prior to exercise and the second Cr dose immediately post-exercise. On non-training days subjects took the supplement *ad libitum*. The placebo group consumed $202.8 \text{ g} \cdot \text{d}^{-1}$ of glucose polymer (101.4 g × 2 daily) for 5 days prepared and administered in an identical fashion to the Cr supplement. Both supplements had similar taste, texture and appearance and were placed in generic packets to ensure double-blind administration.

Subjects were instructed to follow their normal diet (apart from the extra carbohydrate (CHO) contained in the experiment drinks) and to weigh and record all food and drink consumed. Subjects were also requested to eliminate

caffeine and caffeine-containing foods from their diet to minimize the possible inhibitory effects of caffeine on the ergogenic effect of Cr (Vandenbergh *et al*, 1996). Throughout the duration of the experiment, subjects were encouraged to maintain their normal training habits. At the end of the experiment, all subjects gave verbal assurance that they had complied with these instructions. Subjects completed 7 separate 24-hour urine collections. The first was started on the day preceding supplementation (baseline), then continued through the 5 days of supplementation and finished on the day following supplementation.

Procedures

Subjects reported to the laboratory on the morning of testing after a standardized meal and having refrained from alcohol intake, caffeine intake and strenuous exercise the day before. Following the measurement of height and body mass, percentage body fat, fat free mass and total body water (TBW) were measured (Bodystat-1500 Bioimpedance analyzer, Bodystat Ltd., Isle of Man) using a standard bioimpedance technique (Lukaski *et al*, 1985; Van Loan, 1990). The measurements were taken while the subjects lay comfortably in a supine position on a non-conductive surface, with their arms and legs slightly abducted. Before the start of the isometric bench-press test, all subjects underwent a standardized warm-up consisting of 5 min of arm cranking at 25 Watts, followed by a series of stretches with an emphasis on stretching the musculature

associated with the bench-press manoeuvre.

Each subject then performed five consecutive maximal isometric bench-presses. A padded bench was positioned over a calibrated force platform (Kistler type 9281B, Kistler Instruments Corporation, Switzerland) so that the force platform was directly under the subject's shoulders. A weight stand of adjustable height was positioned on either side of the bench, and a 1 m bar was laid across the stands and permanently fixed in this position. Subjects were required to position themselves on the bench with their elbows at 90° flexion and with their hands positioned no more than 81 cm apart. Each subject was asked to assume a comfortable pressing position on the bench. The subject's hand, head and the stand height positions were noted and the subject was required to reproduce the same position on each testing occasion. For each isometric bench-press, subjects were given a 5 s count-down and told to press against the bar as hard as possible for 20 s, a duration that has been shown to deplete PCr stores by ~ 98 % (Tesch *et al*, 1989). The force exerted against the bar was transmitted by the bench to the force platform in the vertical plane, and the peak, total force (area under the curve) and fatigue index (the percent decline in force production within each 20 s period) for each bench-press was calculated using the Kistler software provided (Kistler BioWare, Version 2.22, Kistler Instruments Corporation, Switzerland). This manoeuvre was repeated a further four times, with 2 min recovery periods. This recovery period was adopted in light of the findings by Greenhaff *et al*

(1994) of increased rate of PCr rephosphorylation during the second minute of recovery from intense muscular contractions following Cr supplementation. Subjects adopted the "ready" position in the last minute of each recovery period. All post-supplementation testing was carried out at the same time of day and in the same manner. Consumption of water (500 ml) was permitted during each bench-press test. Room temperature was maintained between 20-24° C.

Data Analysis

Data were expressed as the mean \pm s.d. or median (range), following a test for the normality of distribution. Statistical analysis was carried out using two factor ANOVA for repeated measures, followed by paired *t*-test or two-sample *t*-test, as appropriate. Statistical significance was declared at $P < 0.05$.

RESULTS

Dietary Analysis

During the experimental period, the daily diet of the Cr group comprised $13.4 \pm 2.5 \text{ MJ}\cdot\text{d}^{-1}$, of which $59 \pm 6\%$, $27 \pm 6\%$, $14 \pm 4\%$ and $0 \pm 0\%$ of energy intake was in the form of CHO, fat, protein and alcohol, respectively. The daily diet of the placebo group comprised $13.3 \pm 1.7 \text{ MJ}\cdot\text{d}^{-1}$, of which $59 \pm 5\%$, $25 \pm 6\%$, $14 \pm 4\%$ and $2 \pm 3\%$ of energy intake was in the form of CHO, fat, protein and alcohol, respectively.

Urinary Analyses

In the placebo group, Crea excretion over the six days was not different from baseline. In the Cr group, Crea excretion increased from $1.6 \pm 0.4 \text{ g}\cdot\text{d}^{-1}$ on the first day to $4.0 \pm 1.1 \text{ g}\cdot\text{d}^{-1}$ on the final day of supplementation. Daily Cr excretion was therefore corrected for Crea excretion. Urinary Cr excretion also increased during the supplementation period in the Cr group. Estimated Cr uptake was greatest on the first day of supplementation (15 (7-20) g) and was lowest on the final day of supplementation (7 (-4-15) g) (median (range)). The estimated Cr uptake was calculated by subtracting the total Cr excreted

(corrected for Crea excretion) from the total amount supplemented per day. Of the 20 g of Cr administered each day, 75 (33-100) % was retained on the first day of supplementation and 34 (-18-77) % on the last day of supplementation. The total amount of Cr retained over the supplementation period was 45 ± 18 g of the total supplemented dose (i.e. 100 g), with an estimated increase in intramuscular Cr concentration of 43 (13-61) $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$ (based on an estimated muscle mass of 40% of body mass and an average muscle water of 77% of wet weight; Bergstrom *et al* (1971)). In the placebo group, no Cr was detected in the urine during the experiment. Out of the 21 subjects in the Cr group, 4 subjects were classified as "non-responders" (≤ 21 $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$ increase following Cr supplementation) and the remaining 17 subjects were classed as "responders" (≥ 32 $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$). The estimated Cr uptake for the "responders" group was 51 (32 - 61) $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$ compared to an estimated Cr uptake of 14 (13 - 21) $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$ for the "non-responders". These two distinct groups are evidence of an "ergogenic threshold". These estimated Cr uptakes are very similar to those measured by Greenhaff *et al* (1994). In that study, the "non-responders" had a Cr uptake of about 10 $\text{mmol}\cdot\text{kg}^{-1}$ dry weight muscle and all but one of the "responders" had a Cr uptake greater than 25 $\text{mmol}\cdot\text{kg}^{-1}$ dry weight muscle. Similarly, in the present experiment, all but one "non-responder" had a Cr uptake of about 13 $\text{mmol}\cdot\text{kg}^{-1}$ dry weight muscle and all "responders" had a Cr uptake

above 30 mmol·kg⁻¹ dry weight muscle.

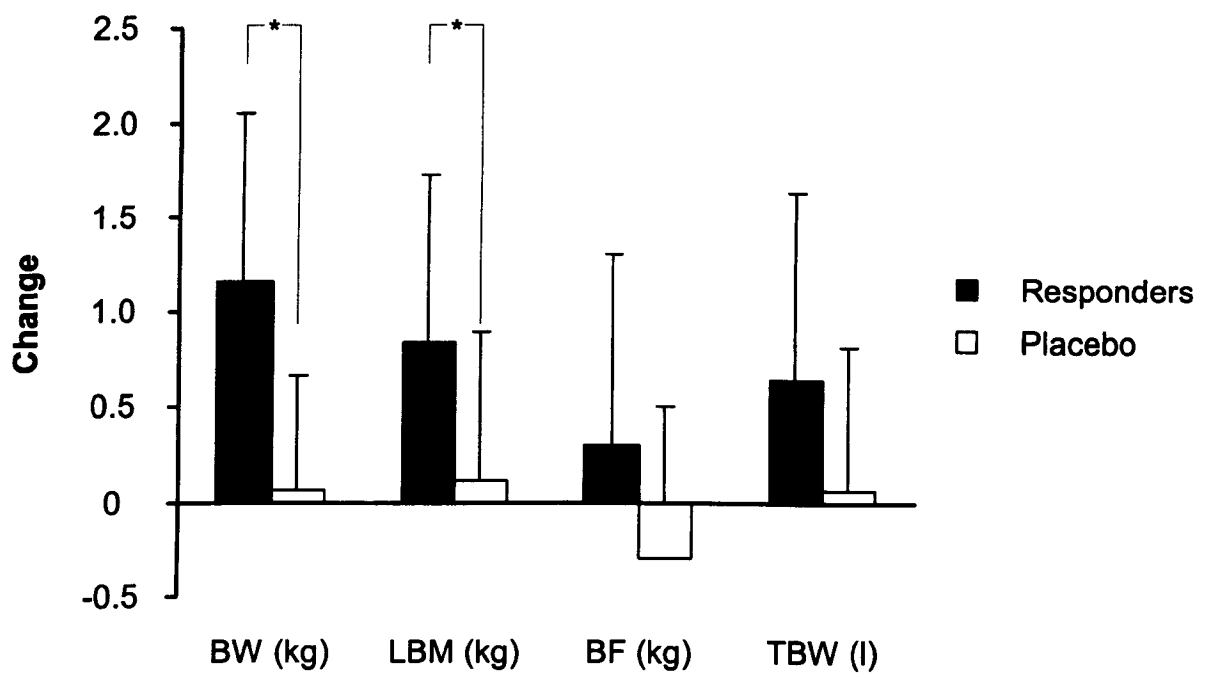
Physical Characteristics

The physical characteristics of the two groups of subjects were similar before supplementation (Table 3.1). In the Cr group, body mass increased significantly from 84.1 ± 8.2 kg to 85.1 ± 8.0 kg following supplementation (P<0.001), with no change in the placebo group (80.1 ± 7.3 kg to 80.2 ± 7.1 kg, P=0.76). The magnitude of change in body mass was significantly greater in the Cr group compared to the placebo group (P=0.003). Absolute and percentage body fat and TBW were not different between groups; however there was a significant increase in TBW in the "responders" over time (49.9 ± 4.3 L to 50.6 ± 4.9 L, P=0.019).

As with the Cr group as a whole, there was a significant increase in body mass in the "responders" to Cr following supplementation (84.1 ± 8.6 kg to 85.3 ± 8.3 kg, P<0.01). The gain in body mass over the supplementation period was also significantly greater (P<0.01) in the "responders" compared to the placebo group (Fig. 3.2). The change in FFM over the supplementation period was significantly greater (P=0.038) in the "responders" compared to the placebo group (Fig. 3.2).

Figure 3.2

Changes in body weight (BW), lean body mass (LBM), fat mass (BF) and total body water (TBW) (mean \pm s.d.) in the "responders" and placebo supplemented groups. * indicates a significantly greater increase in the "responders" group compared to the placebo group



Body mass for the "non-responders" did not increase following Cr supplementation (84.2 ± 7.9 kg to 84.2 ± 7.4 kg, $P=0.94$). The gain in body mass was thus significantly greater ($P=0.017$) in the "responders" compared to the "non-responders". There was no significant increase in average body fat for the "responders" or placebo group post supplementation (i.e. 12.2 ± 3.9 kg to 12.6 ± 3.8 kg, $P=0.237$ and 10.9 ± 3.8 kg to 10.8 ± 3.7 kg, $P=0.788$, respectively). Figure 3.2 shows less than 0.3 kg non-significant increase in body fat for the "responder" group and a non-significant decrease in body fat of 0.3 kg in the placebo group, both are within day to day measurement variation.

Isometric Bench-press Performance

Peak force and total force were not significantly different between the Cr and placebo groups prior to supplementation. In both groups, there was a significant decrease in peak force over the 5 repetitions during both the pre-supplementation and post-supplementation bench-press tests. There was a non-significant tendency for the magnitude of change (i.e. post-supplementation minus pre-supplementation) in peak force and total force to be significantly greater in the Cr group compared to the placebo group ($P=0.054$ and $P=0.078$ respectively). However, when this analysis was repeated after removing the "non-responders" from the Cr group, the magnitude of change in peak force and total force was significantly greater in the "responders" compared to the placebo

group ($P=0.003$ and $P<0.01$) (Fig. 3.3). The percent decline in force production within each 20 s period (fatigue index) for each bench-press was not significantly different when comparing the pre- to the post-supplementation values in either group, or after excluding the "non-responders" from the Cr group.

Correlations

A significant negative correlation was found between estimated Cr uptake and training experience in the Cr group ($r=-0.68$, $n=21$, $P=0.001$) (Fig. 3.4a). Subjects in both the Cr group and the placebo group had, on average, 5 ± 2 years heavy resistance training experience ($P=0.22$). Estimated Cr uptake was also positively correlated with the change (Δ) in body mass over the supplementation period ($r=0.55$, $n=21$, $P<0.01$) (Fig. 3.4b). Estimated Cr uptake was significantly correlated with the increase (pre to post) in total force for the first 4 repetitions (Repetition 1: $r=0.53$, $P=0.013$; Repetition 2: $r=0.47$, $P=0.033$; Repetition 3: $r=0.44$, $P=0.044$; Repetition 4: $r=0.49$, $P=0.026$). There was also a significant positive correlation between the magnitude of increase in total force over the 5 repetitions (total change in force for each repetition added together) and estimated Cr uptake ($r=0.508$, $n=21$, $P=0.019$) (Fig. 3.4c). No significant association was found when protein intake was correlated against estimated Cr uptake ($P=0.79$).

Figure 3.3

Change in peak force (top panel) and total force (bottom panel) in the Cr ("responders" and "non-responders") and placebo supplemented groups. * indicates a significantly greater increase in the "responders" group compared to the placebo group

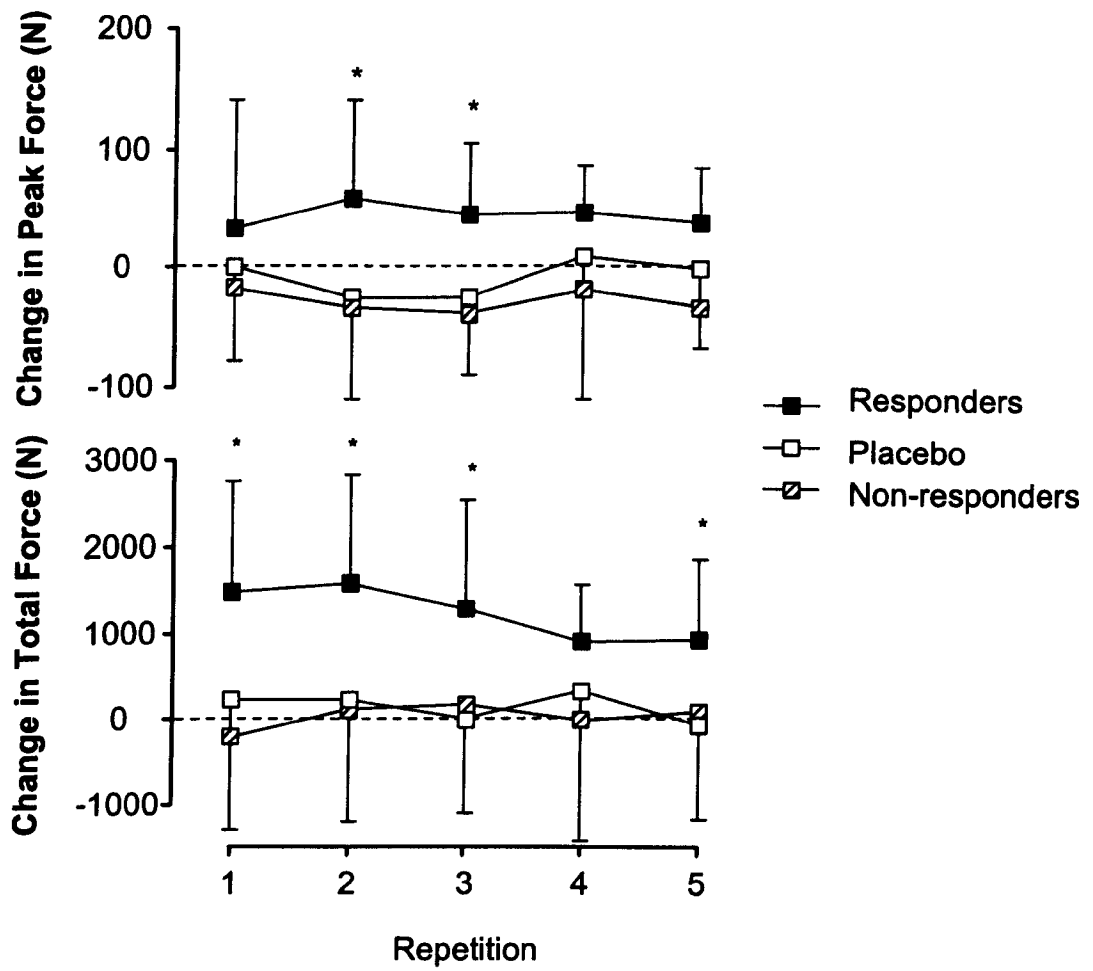
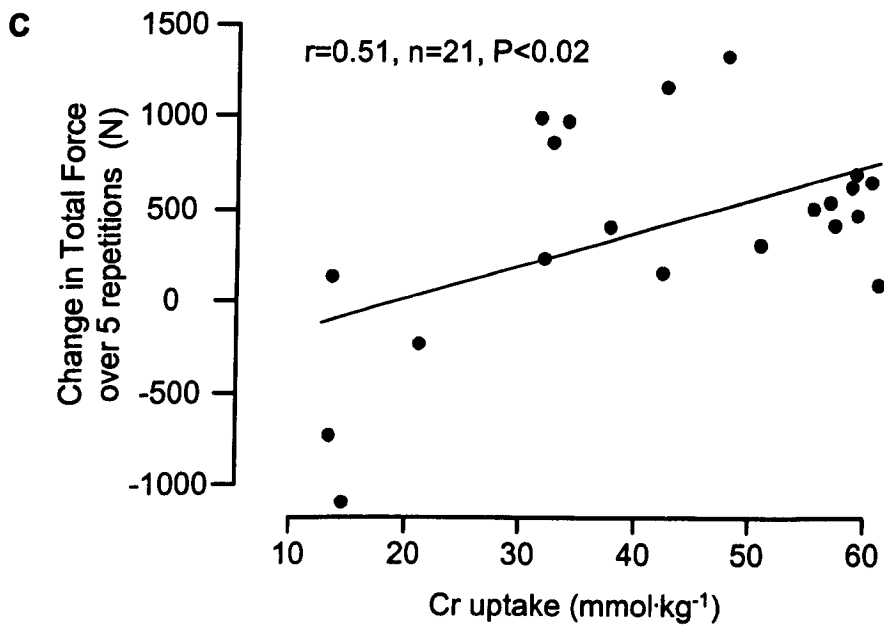
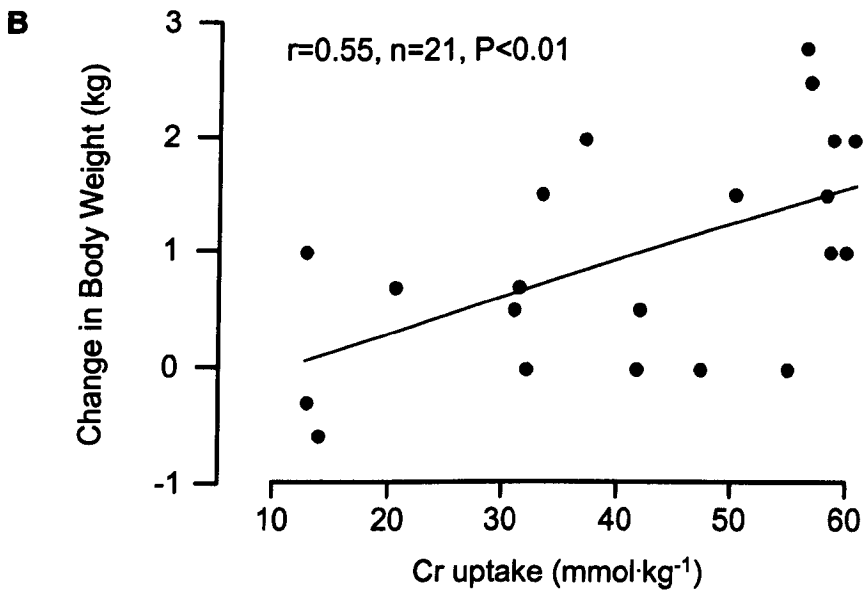
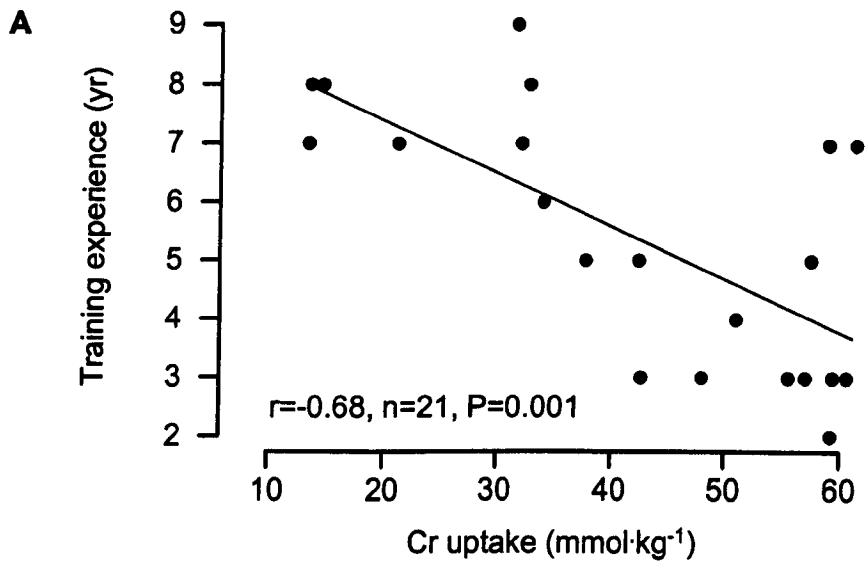


Figure 3.4

Correlations between estimated Cr uptake and training experience (A), change in body weight (B) and change in total force (C) in the Cr group



Side Effects

In general, subjects tolerated the supplementation protocol well, with no reports of gastrointestinal distress or muscle cramping. Three subjects (i.e. 2 subjects in the Cr group and 1 subject in the placebo group) reported experiencing mild headaches, possibly due to the high glucose concentration of the ingested supplements.

DISCUSSION

In the present experiment, 5 days of Cr supplementation significantly increased peak isometric force and total force during repeated 20 s isometric bench press exercise in a group of 17 "responders" compared to the placebo group (Figure 3.3). As muscle biopsies were not obtained in this Experiment, one can only speculate on the potential mechanisms for this improvement in performance following Cr supplementation. Nevertheless, increased PCr availability and PCr resynthesis during recovery from maximal exercise of this type are the most plausible as muscle fatigue has previously been associated with a depletion of muscle PCr stores (Hultman *et al*, 1990; Tesch *et al*, 1989). Cr supplementation has the potential to increase the basal levels of PCr and, by doing so, to delay the onset of muscle fatigue. A number of previous studies support this view (Balsom *et al*, 1995; Greenhaff *et al*, 1994).

Since the seminal work of Harris *et al* (1992) and Greenhaff *et al* (1994), many investigators have tested the hypothesis that strength, power and/or work performed during repeated sets of maximal dynamic contractions can be improved by increasing total muscle Cr concentration by Cr ingestion. However, not all studies have reported an ergogenic effect. Of the 55 pertinent papers published to date (to our knowledge and not including abstracts), 40 have demonstrated an ergogenic effect. It is interesting to note that Greenhaff *et al*

(1994) found a substantial increase in total muscle Cr concentration only in subjects with a pre-supplementation total muscle Cr concentration of the order of 120 mmol·kg⁻¹·dry muscle weight or less, and that these same individuals demonstrated an accelerated rate of PCr resynthesis during the second minute of recovery from intense electrically-evoked contractions of the vastus lateralis.

Greenhaff *et al* (1994) and Casey *et al* (1996) subsequently showed an ergogenic effect of Cr supplementation when the post-supplementation increase in intramuscular [Cr] exceeded 20 mmol·kg⁻¹·dry muscle weight. For example, Casey *et al* (1996) reported that Cr supplementation produced a 23.1 ± 4.7 mmol·kg⁻¹·dry muscle weight increase in [Cr] and an increase in peak and total work produced during two bouts of 30 s maximal isokinetic cycling. Similarly, Maganaris & Maughan (1998) showed that Cr supplementation (10 g Cr·d⁻¹ for 5 days) increased the estimated muscle [Cr] by about 30 mmol·kg⁻¹·dry muscle weight and increased isometric force generating capacity and isometric endurance. In contrast, however, Snow *et al* (1998) found only a small increase in muscle [Cr] following 30 g of Cr·d⁻¹ for 5 days (i.e., 11.7 ± 2.4 mmol·kg⁻¹·dry muscle weight) and no significant improvement in sprint-exercise performance. A similar outcome was reported more recently by Finn *et al* (2001) for 4x20 s all-out sprint performance in 8 endurance trained cyclists following 5 days of Cr supplementation. They too found only a small increase in muscle [Cr] (16.2

mmol·kg⁻¹·dry muscle weight), with 3 out of the 8 subjects increasing [Cr] by less than 10 mmol·kg⁻¹·dry muscle weight. It should be pointed out that these authors could be making a Type II error (Tarnopolsky & MacLennan, 2000) consequent both to the small sample size and the low Cr retention that was particularly marked in 3 of their 8 subjects.

These several observations emphasize the importance of recognizing that substantial individual differences can occur in intramuscular Cr uptake following Cr supplementation. The classification of subjects into "responders" and "non-responders" (Greenhaff *et al*, 1994; Casey *et al*, 1996) is suggestive of what might be termed an "ergogenic threshold" for Cr uptake of about 20 mmol·kg⁻¹·dry muscle weight, as proposed by Greenhaff *et al* (1994) and Casey *et al* (1996). Greenhaff *et al* (1994) showed an increased rate of PCr resynthesis during recovery following Cr ingestion in subjects whose muscle Cr concentration increased by on average 20 mmol·kg⁻¹·dry muscle weight, but conversely subjects whose muscle Cr concentration increased by < 10 mmol·kg⁻¹·dry muscle weight following Cr supplementation showed very little or even a slower rate of PCr resynthesis during recovery. Our findings provide support for this contention. Only when the 4 "non-responders" (in whom the increase in intramuscular [Cr] was estimated to be ≤ 21 mmol·kg⁻¹·dry muscle weight) were excluded from the Cr group did the improved isometric bench-press performance clearly emerge,

both in terms of peak force and total force. Furthermore, the finding of a significant correlation between estimated Cr uptake and delta total force in repetitions 1 - 4 would suggest that subjects with the greatest Cr uptake had the greatest performance benefit, and this is in agreement with previous published work (Casey *et al*, 1996). While these findings provide evidence consistent with an ergogenic threshold, assigning a specific threshold value is not possible as Cr uptake was only estimated in the present experiment. Nevertheless, these estimated Cr uptake values are very similar to those measured by Greenhaff *et al* (1994).

One explanation for the two distinct groups (i.e. "responders" and "non-responders") with regard to Cr uptake may be the varying amount of intramuscular Cr prior to supplementation. This might reflect, for example, a low habitual dietary intake of Cr in the "responders" and/or conversely, a high dietary intake of Cr in the "non-responders". Whether this was the case in these particular subjects cannot be established. While our subjects carried out a weighed intake of food, it was not possible to meaningfully estimate the dietary Cr content as the amount of Cr in each item of food is dependent on many factors including food preparation. Even so, one might reasonably expect that subjects with a high protein intake might also have a high Cr intake. However, we found no significant correlation between protein intake and estimated Cr uptake

($P=0.79$). Also, protein intake was not different in the "responders" and "non-responders".

Another possible explanation could be the strength-training status of our subjects. MacDougall *et al* (1977) have reported that only 5 months of heavy resistance-training can increase resting muscle [Cr] by 39% and [PCr] by 22%. Our subjects had 5 ± 2 years of heavy resistance training experience which could predispose them to high resting levels of intramuscular Cr and PCr. Variability in training status may therefore be another factor responsible for the conflicting results reported in the literature. Interestingly, a significant negative correlation was found between training experience and estimated Cr uptake (Fig. 3.4a). This is an area that we feel needs further investigation.

Although other studies have found no significant difference in body mass following short-term Cr supplementation ($20 - 30 \text{ g}\cdot\text{d}^{-1}$ for 5 - 7 days) (Grindstaff *et al*, 1997; Steenge *et al*, 1998; Terrillion *et al*, 1997), the majority of studies have produced increases ranging from 0.6 - 1.8 kg following short-term Cr supplementation (Earnest *et al*, 1995; Green *et al*, 1996; Greenhaff *et al*, 1994; Maganaris & Maughan, 1998; Terrillion *et al*, 1997). Considering the short time course of this increase in body mass, some investigators have attributed these increases to increases in TBW. For example, Hultman *et al* (1996) found a 0.6 L decline in urinary volume following acute Cr supplementation ($20 \text{ Cr g}\cdot\text{d}^{-1}$ for 6

days) and therefore attributed the increase in body mass to Cr-stimulated water retention. These authors also noted that the time course of urinary volume changes paralleled that of muscle Cr uptake. Furthermore, an increase in both total body and intracellular water was demonstrated by Ziegenfuss *et al* (1998) with acute Cr ingestion ($0.35 \text{ g}\cdot\text{kg fat free mass}\cdot\text{d}^{-1}$ for 3 days), with the increase in TBW accounting for approximately 90% of the acute gain in body mass. It remains to be determined whether this increase in water is associated with an increase in protein synthesis. The balance of available evidence from human performance studies using Cr supplementation and more direct evidence from animal *in vivo* and *in vitro* experiments would support the notion that increasing Cr availability may indeed increase protein synthesis (Francaux & Poortmans, 1999; Ingwall *et al*, 1974; Kreider *et al*, 1998). In the present Experiment, supplementation with Cr increased body mass ($84.1 \pm 8.6 \text{ kg}$ pre-supplementation to $85.3 \pm 8.3 \text{ kg}$ post-supplementation), with the mean increase in the "responder" group ($1.2 \pm 0.9 \text{ kg}$) being significantly greater ($P < 0.01$) than that of the placebo group ($0.1 \pm 0.6 \text{ kg}$) (Fig. 3.2). The increase in body mass cannot be explained by the increases in TBW alone as a result of Cr stimulated water retention, as there was no significant increase in TBW as expressed as percentage of body mass. Despite a significant increase in TBW in absolute terms in the "responders" group ($49.9 \pm 4.3 \text{ L}$ to $50.6 \pm 4.9 \text{ L}$, $P = 0.019$), if the relative volume of TBW remains constant (as in the present Experiment), the gain in body mass may not be attributed to water retention. The increase in absolute

TBW seen in this study following Cr supplementation may be indicative of intracellular water that normally accompanies dry matter growth. Francaux and Poortmans (1999) found similar results and interpreted their findings in a same manner. Cr supplementation also promoted significantly greater gains in FFM in the "responder" group compared to the placebo group ($P=0.038$). Resolution of this issue requires additional research using more precise and invasive methods.

CONCLUSION

The results of this Experiment suggest that 20 g Cr·d⁻¹ for 5 days did not result in a significant increase in peak force or total work during repeated isometric contractions in resistance-trained individuals. However, this was due to the "non-responders" in the Cr group masking the effects of the remaining group. When the Cr group was considered with only "responders" to Cr in the group, Cr supplementation resulted in significant increases in peak force and total force.

CHAPTER FOUR

(Experiment 2)

Effects of Creatine on Body Composition and Strength Gains after Four Weeks of Resistance-training in previously Non-resistance-trained Humans

INTRODUCTION

The seminal work of Harris *et al* (1992) and Greenhaff *et al* (1994) instigated a number of studies that tested the hypothesis that strength, power and/or work, performed during repeated sets of maximal dynamic contractions, may be improved following an increase in total muscle [Cr] ([TCr]) by Cr ingestion. Not all studies, however, have reported an ergogenic effect. Of the 55 pertinent papers published to date (to our knowledge and not including abstracts), 40 have demonstrated an ergogenic effect. The majority of the conflicting findings may be attributed in part to the failure of many researchers to acknowledge the considerable inter-individual variation in muscle Cr uptake following Cr supplementation. Subjects were not, therefore, differentiated into "responders" and "non-responders" ($< 21 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{dry muscle weight increase}$ following Cr supplementation; Greenhaff *et al* (1994)) on the basis of measured or estimated Cr uptake. In order to examine whether Cr supplementation can enhance performance, it is essential that experiments are designed to optimise Cr uptake and therefore potential to improve performance.

Over the years, numerous strategies have emerged aimed at enhancing Cr uptake with Cr supplementation. The early work of Harris *et al* (1992) is one of only a few studies to examine whether Cr uptake by skeletal muscle can be increased by exercise. Harris *et al* (1992) employed 1 hour of one-legged cycling

exercise per day for 4 - 7 days and demonstrated enhanced Cr uptake in the exercising leg by 54% whilst little effect was observed in the non-exercising leg. The precise mechanism(s) for the exercise-induced increase in muscle Cr uptake is still unknown, although an increase in blood flow to the exercising muscle and/or changes in the transport kinetics of Cr across the sarcolemma were suggested by the authors as the most plausible explanations.

An additional strategy to show enhanced Cr uptake is the ingestion of carbohydrate in combination with Cr. Ingestion of Cr, in combination with a carbohydrate-containing solution, resulted in a 60% greater increase in muscle [Cr] than when ingesting Cr on its own (Green *et al*, 1996). While the explanation for this carbohydrate-induced increase in muscle Cr uptake remains unclear, this response is most likely to be the result of an insulin-mediated increase in muscle Cr transport, rather than an effect on Cr delivery (Green *et al*, 1996; Steenge *et al*, 1998).

Training status is another factor with the potential to influence Cr uptake. Experiment 1 revealed a significant negative correlation between resistance training experience/history and estimated muscle Cr uptake (Fig. 3.4a), suggesting the greater the resistance-training experience of the subjects, the lower the Cr uptake with Cr supplementation. This notion is also supported by the early work of MacDougall *et al* (1977), showing that only 5 months of heavy

resistance-training increased resting muscle [Cr] by 39% and [PCr] by 22%. Heavy resistance training experience could therefore predispose subjects to high baseline levels of intramuscular Cr and PCr and, therefore, to a reduced potential for Cr uptake. If correct, the likelihood of obtaining a large Cr uptake and, hence, greater performance gains will be increased in non-resistance-trained subjects.

In light of the above, Experiment 2 was designed with these strategies in mind to order to help optimise muscle Cr uptake and, hence, the potential to enhance strength. The purpose of this experiment, therefore, was to investigate the effects of 4 weeks of Cr supplementation after four weeks of resistance-training in previously non-resistance-trained humans on isokinetic, isometric and isotonic strength.

METHODS

Subjects

Twenty healthy non-resistance trained males (Table 4.1) from whom written informed consent had been obtained, volunteered to take part in the present Experiment which was approved by the local ethics committee; one subject was unable to comply with all experimental procedures and was therefore excluded. No subject had a record of cardiovascular or respiratory disease and none had subjective evidence of musculoskeletal injury. Subjects were recruited on the basis that they were physically active but not engaged in a structured weight-training programme for at least 6 months prior to the start of the experiment and had not supplemented with Cr for at least 8 weeks before the experiment. The subject's eligibility was assessed by interview prior to their informed consent for participation in the experiment.

Experimental Design

Prior to the commencement of the experimental trials, subjects visited the laboratory on at least two occasions in order to become familiar with the isokinetic dynamometer, leg press and related protocols. Familiarization trials were carried out until the variability of two consecutive performances was

Table 4.1: Physical characteristics of the two groups of subjects

	Placebo Group (n=10)		Creatine Group (n=9)	
	Baseline	Post-loading	Baseline	Post-loading
Age (yr)	21 ± 1	-	20 ± 3	-
Height (m)	1.78 ± 0.04	-	1.75 ± 0.05	-
Body mass (kg)	76.2 ± 10.1	77.0 ± 9.8 *	70.3 ± 10.5	71.7 ± 11.2 *
Total Body Water (L)	41.5 ± 3.7	41.8 ± 3.6	41.0 ± 4.7	41.7 ± 4.9 *
Extracellular Water (L)	18.9 ± 1.5	19.1 ± 1.5	18.8 ± 2.1	19.0 ± 2.2
Intracellular Water (L)	22.6 ± 2.2	22.7 ± 2.1	22.2 ± 2.5	22.8 ± 2.7 *
				23.0 ± 2.8 *

Values are presented as the mean ± s.d.

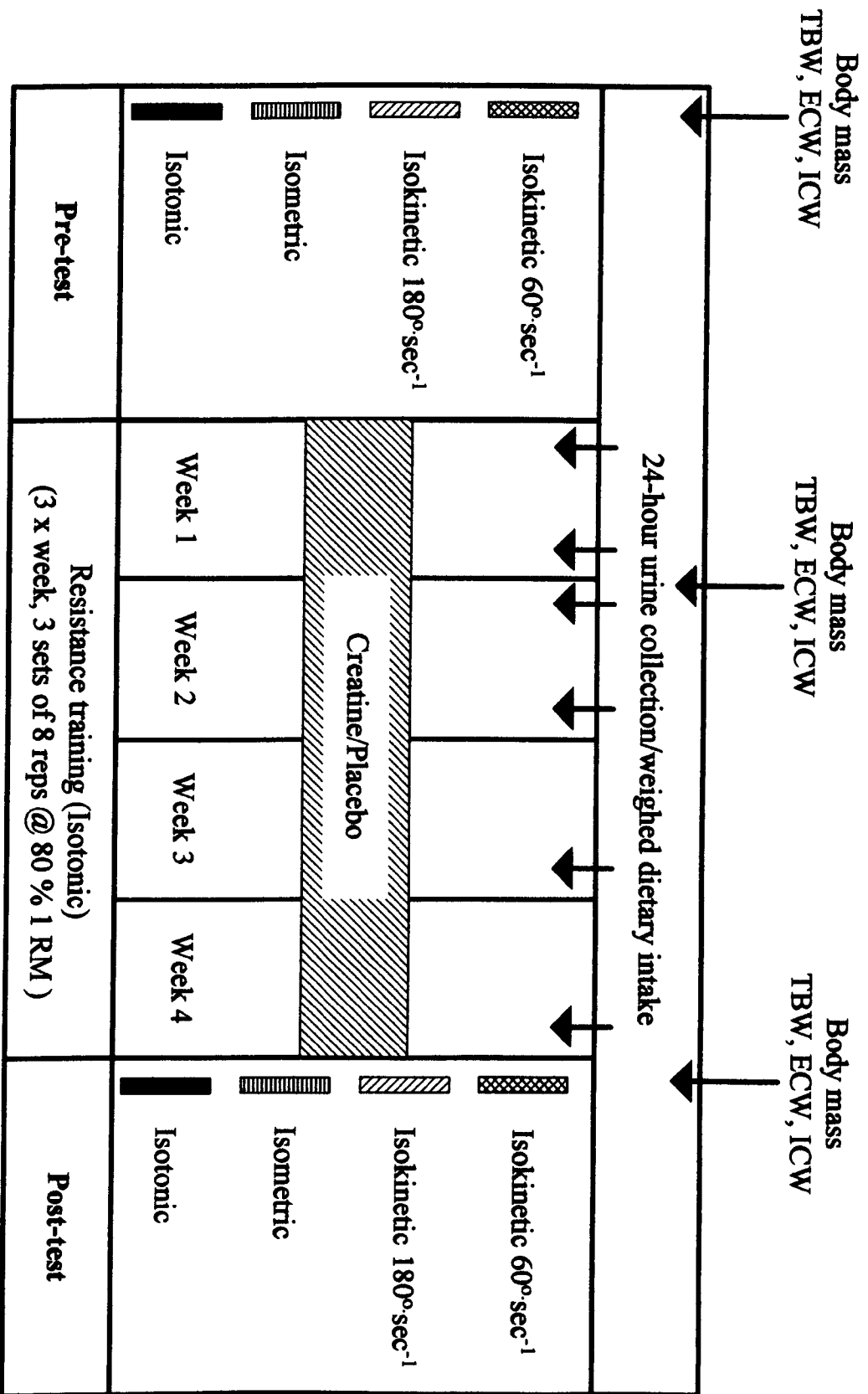
* Indicates a significant difference from baseline

within 5% for peak isokinetic force at a speed of $60^{\circ}\cdot\text{s}^{-1}$. The test-retest reliability of both the isokinetic and isometric assessments revealed a high intra-class correlation (ICC) for all strength measures (Isokinetic force ICC = 0.92 ($60^{\circ}\cdot\text{s}^{-1}$) and 0.89 ($180^{\circ}\cdot\text{s}^{-1}$), Isometric force ICC = 0.93; these test-retest reliability values are based on subjects having each undergone two familiarization tests). Subjects were assigned in a double blind fashion to either a Cr group or a placebo group, based on the final familiarisation results: subjects were matched into pairs on the average of their peak force obtained during the $60^{\circ}\cdot\text{s}^{-1}$, $180^{\circ}\cdot\text{s}^{-1}$ isokinetic and isometric leg extension and randomly assigned so that one member of each pair was in the Cr group and the other in the placebo group. Following the familiarisation period, all subjects performed two tests carried out at least 28 days apart. The first test was conducted 48 hours after the final familiarisation trial, and the final test was conducted 48 hours after the final training session. The 4-week supplementation and training period for both groups started on the day after the first test and finished the day before the second test. The experimental design is shown in Figure 4.1.

The Cr group ingested $22.8\text{ g}\cdot\text{d}^{-1}\text{ Cr}\cdot\text{H}_2\text{O}$ (equivalent to 5 g Cr \times 4 times daily) for the first 7 days. This loading phase has been shown to increase resting muscle PCr levels (Harris *et al*, 1992, Greenhaff *et al*, 1994, Hultman *et al*, 1996). From day 8, subjects consumed $5.7\text{ g}\cdot\text{d}^{-1}\text{ Cr}\cdot\text{H}_2\text{O}$ (equivalent to 5 g Cr daily) for the

Figure 4.1

The experimental design for Experiment 2



remaining 21 days. This maintenance dose was selected on the basis of published work by Hultman *et al* (1996) showing $2 \text{ g}\cdot\text{d}^{-1} \text{ Cr}\cdot\text{H}_2\text{O}$ was adequate in maintaining elevated muscle PCr stores in subjects not involved in strenuous exercise. As the subjects in the present Experiment were training three times a week at high intensities, it was decided to increase the maintenance dose to $5 \text{ g}\cdot\text{d}^{-1} \text{ Cr}\cdot\text{H}_2\text{O}$ in an attempt to maintain muscle PCr stores. Each supplement consisted of 5.7 g of Cr·H₂O and 35 g of glucose polymer made up in 500 ml of warm to hot water. Dissolving Cr in warm to hot water prevented any detectable formation of creatinine (Crea) and no parts of the supplement remained undissolved. The addition of glucose to the Cr has been shown to significantly enhance the uptake of Cr (Green *et al*, 1996; Steenge *et al*, 1998). Subjects were instructed to ingest the supplements at equal intervals throughout the day. On training days, subjects ingested one Cr supplement 1 hour prior to exercise and another Cr supplement immediately after exercise. The pre and post-training supplements were prepared and administered by the supervising investigator. The placebo group consumed $160 \text{ g}\cdot\text{d}^{-1}$ of glucose polymer ($40 \text{ g} \times 4$ times daily) for the first 7 days, followed by 40 g a day for the subsequent 21 days. The placebo group followed the same procedure as the Cr group with regard to the preparation of the supplements. Both supplements had similar taste, texture and appearance and were placed in generic packets to ensure double-blind administration.

Subjects completed 24 hour urine collections for the day preceding the start of supplementation (baseline), the first day of supplementation (start of loading), the final day of loading and once a week for the following 3 weeks. Subjects were also required to carry out a weighed intake of food at set intervals throughout the experimental period (i.e. 3 days prior to the start of the experiment followed by 1 day a week for the 4 weeks of the experiment). Subjects were instructed to follow their normal diet (apart from the extra carbohydrate contained in the experimental drinks) and to weigh and record all food and drink consumed. Digital weighing scales readable to 1 g were used. Subjects were also requested to eliminate caffeine and caffeine containing foods from their diet over the loading phase to minimize the possible inhibitory effects of caffeine on the ergogenic effect of Cr (Vanderberghe *et al*, 1996). At the end of the experiment, all subjects gave verbal assurance that they had complied with all instructions.

Procedures

Subjects reported to the laboratory on the morning of testing after having refrained from alcohol, caffeine and strenuous exercise the day before. Following the measurement of each subject's stature and body mass, body water compartments were measured using a Bodystat Multiscan 5000 Bioimpedance analyzer (Bodystat Ltd., Isle of Man). This method allows total body water

(TBW) and extra-cellular water (ECW) to be estimated; from these measurements intra-cellular water (ICW) can also be deduced. The bioimpedance measurements were taken while the subjects lay comfortably in a supine position on a non-conductive surface with their arms and legs slightly abducted. Following the bioimpedance measurement, subjects underwent a standardized warm-up which comprised of 5 min light intensity cycling, followed by a series of stretches with an emphasis on stretching the musculature associated with the leg press and leg extension movements.

Following the standardized warm-up, isokinetic ($60^{\circ}\cdot\text{s}^{-1}$ and $180^{\circ}\cdot\text{s}^{-1}$) and isometric strength was measured during 3 repetitions using a Kin-Com II isokinetic dynamometer (Chattecx Corporation, Chattanooga, USA). Both the right and left knee extensors were tested in random order; with the order being replicated during the post-supplementation tests. The position of the subject on the isokinetic dynamometer was standardized; the anatomical axis of the knee joint was aligned with the rotational axis of the dynamometer by adjusting the seat position and the lever head of the dynamometer. Individual seat length and height were recorded for each subject and used in subsequent tests. Subjects were held in the seat position with Velcro belts around the waist, thigh and lower leg proximal to the ankle, this also allowed for complete isolation of the testing leg. During the measurement, subjects had their arms crossed over their chest while their non-involved leg and upper body was kept stationary. Subjects

were instructed to exert maximal effort throughout the full range of motion during each repetition. Verbal encouragement was given to maximise performance. The same investigator conducted all tests. Subjects performed three maximal isokinetic concentric contractions at two speeds ($60^{\circ}\cdot\text{s}^{-1}$ and $180^{\circ}\cdot\text{s}^{-1}$) with a 15 s rest period between repetitions (Cress *et al*, 1992). The two testing speeds were separated by a 2 min resting period. Having completed the isokinetic testing and following an additional 2 min recovery period, subjects went on to complete three maximal 5 s isometric contractions with a 1 min rest period between repetitions. Keus *et al* (1994) reported that a 5s maximal voluntary isometric contraction provided the subject with ample time for the development of maximal contractile force while minimising the possibilities of any significant fatigue effects. All subjects will be given verbal encouragement throughout the tests and special care was taken in order that all subjects were offered the same instructions and the same degree of encouragement on all visits.

The isokinetic dynamometer was then set up for testing the opposite leg and the same testing order and procedure was repeated (subjects dominant leg was always tested first). Knee extensor strength was evaluated using an isokinetic dynamometer due to the high reliability and reproducibility of this strength assessment (Pincivero *et al*, 1997).

Following a 10 min recovery period, subjects completed a 1 RM on the isotonic leg press to measure the subject's maximal isotonic strength. Subjects began with a warm-up set of ten repetitions at 50% of their 1 RM (determined during their second familiarisation trial); this was followed by a 2-min recovery period. The position of the subject on the leg press machine was standardised; the subject's hip, knee and ankle were set at angles of 80°, 90° and 80° respectively using a goniometer and the foot position on the lifting plate was noted. Following the warm-up set, subjects attempted a 1 RM. Subjects were required to raise and lower the weight in a controlled manner. The lifting weight was increased after each successful lift, until the subject could not lift the weight through the full range of motion. The 1 RM was determined after 3 - 5 attempts in all subjects. All post-supplementation testing was carried out at the same time of day and in the same manner. Consumption of water (500 ml) was permitted during each test. Room temperature was maintained between 20 - 24° C.

Strength Training

Prior to the start of each training session, all subjects underwent a standardised warm-up which comprised light intensity cycling for 5 min, followed by a series of stretches with an emphasis on stretching the musculature associated with the leg press. Subjects then performed one warm-up set of 8

repetitions at 50% of their predetermined 1 RM. Following the warm-up set, subjects attempted to completed 3 sets of 8 repetitions at 80% of their predetermined 1 RM (Baechle *et al*, 2000) with 2 minutes rest between sets (Greenhaff *et al*, 1994). The training weight was progressively increased (~ 5kg) as subjects successfully completed the required number of sets and repetitions. Subjects kept training logs throughout the duration of the Experiment detailing weight, sets and repetitions lifted during each training session. Subjects trained three times a week on non-consecutive days for 4 weeks (i.e. 12 sessions in total). All training sessions were conducted at the same time of day for each subject and were supervised by at least two investigators. Subjects trained in pairs, with subjects matched for strength in order to add a competitive nature to the training. Training sessions lasted on average 50 min (including the 15 min warm-up and 10 min cool-down).

Data analysis

Data were expressed as the mean \pm s.d. following a test for the normality of distribution. Statistical analysis was carried out using two factors ANOVA for repeated measures, followed by Student's *t*-test for paired data and two sample *t*-tests for unpaired data, as appropriate. Total lifting volume was analyzed by ANCOVA to normalize differences between groups in pre-supplementation results using session 1 as the covariate. Pearson correlation analysis was used to

assess the relationship between variables. Statistical significance was declared when $P < 0.05$.

RESULTS

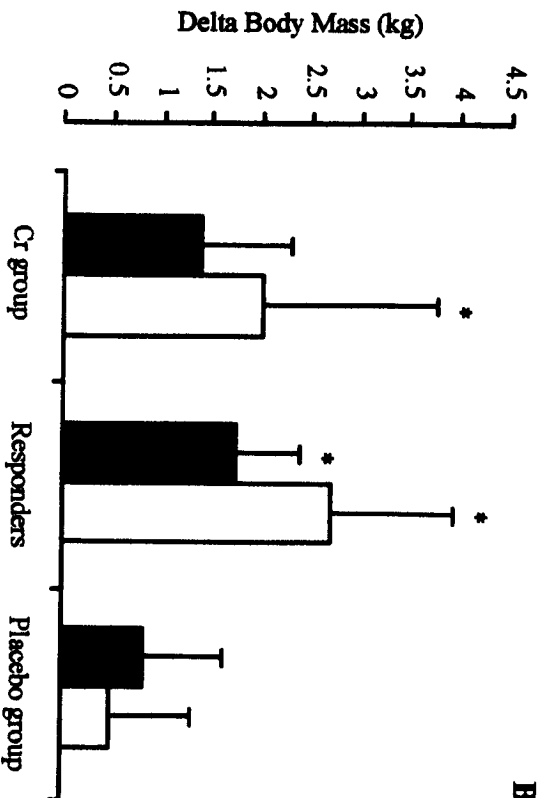
Physical Characteristics

The physical characteristics of the two groups of subjects are presented in Table 4.1. There were no significant differences between groups in body mass or any of the body composition measurements pre-supplementation. In the Cr group, mean body mass increased 1.4 ± 0.9 kg after week 1 (loading phase) and 2.0 ± 1.8 kg after 4 weeks of supplementation and training (maintenance phase). In the placebo group, mean body mass increased 0.8 ± 0.8 kg only after loading. The magnitude of change in body mass was greater in the Cr group over the 4 weeks when compared to the placebo group (Figure 4.2); however, there was no difference following the loading period. Out of the 9 subjects in the Cr group, 2 subjects were classified as "non-responders" based on their urinary and body mass data (i.e. ≤ 0.2 kg increase in body mass) and low Cr retention following Cr supplementation (see *Urinary Analysis* section in results) and the remaining 7 subjects were classed as "responders". This is based on the results from Experiment 1 showing a positive correlation between estimated Cr uptake and change in body mass ($r=0.55$, $n=21$, $P<0.001$) (Figure 3.4b). As with the Cr group as a whole, there was a significant increase in body mass in the "responders" to Cr following loading and maintenance (Table 4.1). The gain in body mass over

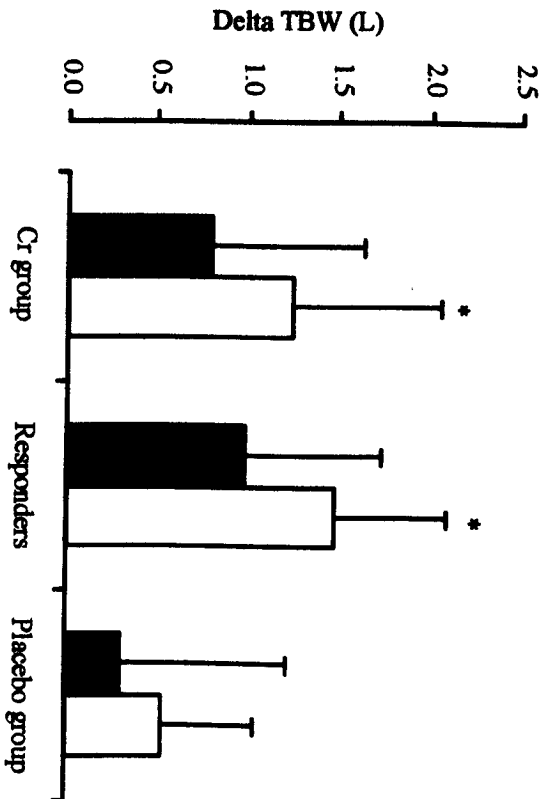
Figure 4.2

Changes in body mass (A), TBW (B), ICW (C) and ECW (D) (Mean \pm s.d.) post-loading and post-maintenance. * indicates a significantly greater increase in the "responders" and/or Cr group compared to the placebo group

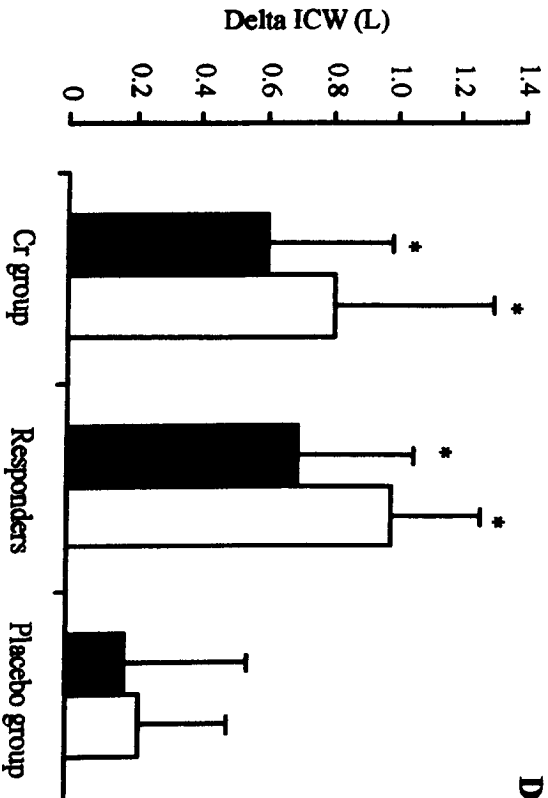
A



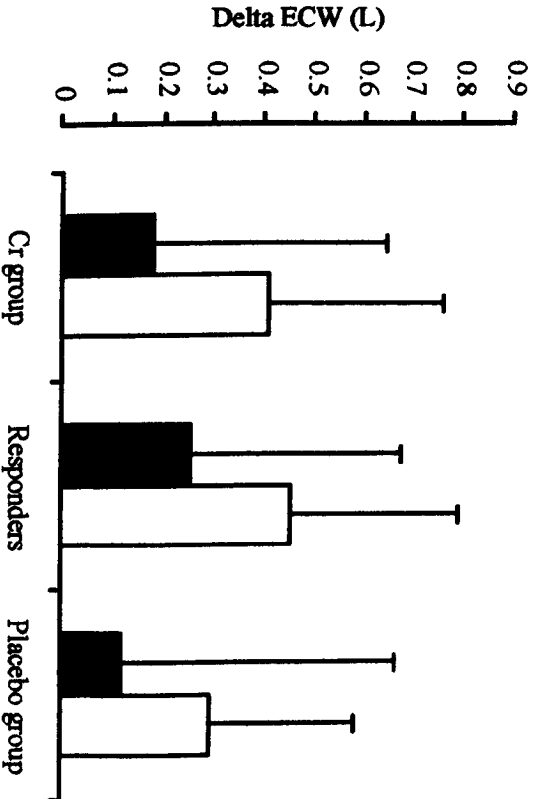
B



C



D



■ Post-loading
□ Post-maintenance

The loading and maintenance period was also significantly greater in the "responders" compared to the placebo group (Figure 4.2).

In the Cr group, TBW and ICW increased after loading and maintenance (Table 4.1). In the placebo group, increases in TBW and ICW were observed only after maintenance. There was no change in ECW in either group after loading; however ECW increased significantly in both groups after maintenance. The magnitude of change in TBW and ICW was greater in the Cr group compared to the placebo group over the 4 weeks of the experiment (Figure 4.2). When the change in TBW and ICW was compared between the "responders" and placebo group, the magnitude of change was significantly greater over the loading period in the "responders" group with regard to ICW and over the maintenance phase in both TBW and ICW.

Dietary Analysis

During the experimental period, the normal daily diet of the Cr group comprised 10.9 ± 2.9 MJ·d⁻¹, of which $55.8 \pm 3\%$, $29.4 \pm 3.8\%$ and $14.8 \pm 1.9\%$ of energy intake was in the form of CHO, fat, and protein, respectively. The normal daily diet of the placebo group comprised 11.0 ± 1.4 MJ·d⁻¹, of which $58.5 \pm 4.9\%$, $27.3 \pm 4.4\%$, and $14.2 \pm 1.9\%$ of energy intake was in the form of CHO, fat, and

protein, respectively. There was no difference between groups in energy intake or diet composition over the duration of the Experiment.

Muscle Strength

Muscle strength was reported by averaging peak torque (i.e. three attempts with the dominant leg and three attempts with the non-dominant leg). Muscle strength was not different between groups prior to supplementation. In the Cr group, average peak torque during isokinetic (both 60 and 180°·s⁻¹) and average peak force isometric concentric knee extensions increased post-supplementation and training compared to baseline; no increase in muscle strength was found in the placebo group (Figure 4.3). The magnitude of change in muscle strength (average peak torque during 60 and 180°·s⁻¹ isokinetic and isometric concentric knee extensions) was not significantly different between groups (P=0.28, P=0.067 and P=0.21, respectively) (Figure 4.4). However, when the analysis was repeated excluding the two subjects classed as "non-responders", the magnitude of change in 180°·s⁻¹ isokinetic force (P=0.029) and isometric force (P=0.036) in the "responders" was greater compared to the placebo group; no difference in 60°·s⁻¹ isokinetic force (P=0.13) (Figure 4.4).

Figure 4.3

Isokinetic ($60^{\circ}\cdot\text{s}^{-1}$ and $180^{\circ}\cdot\text{s}^{-1}$) and isometric force in the Cr group, "responders" and placebo group (mean \pm s.d.) pre- and post-supplementation. * indicates a significantly increase from pre to post

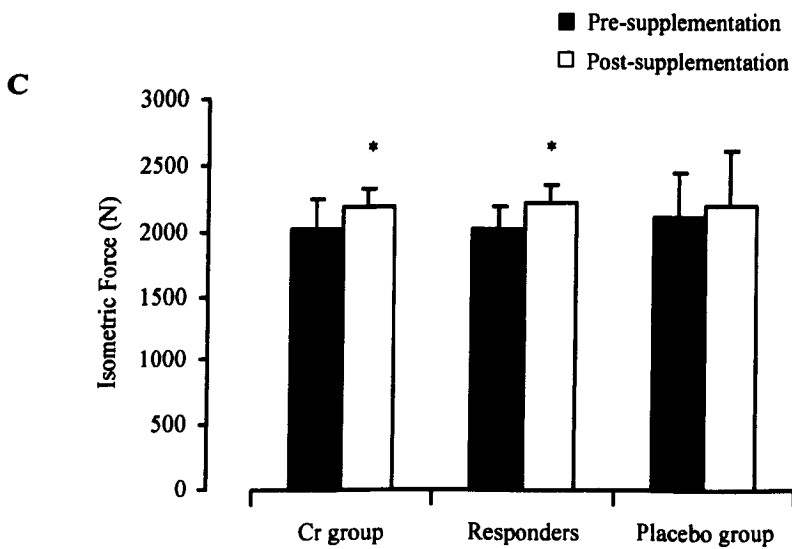
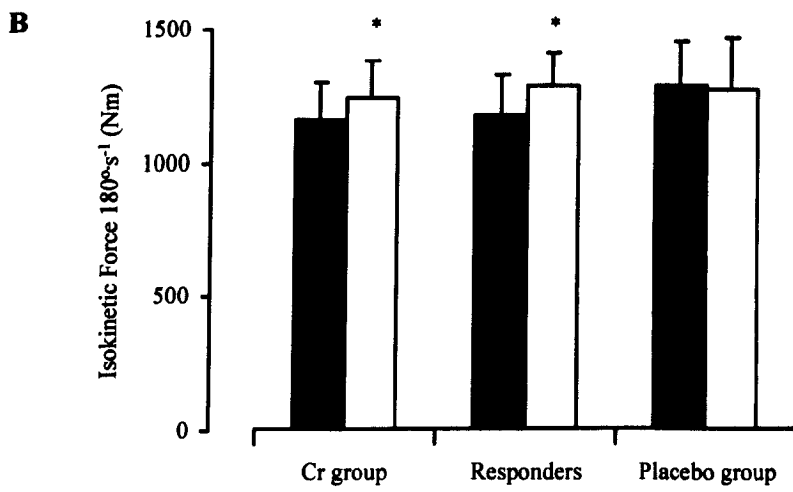
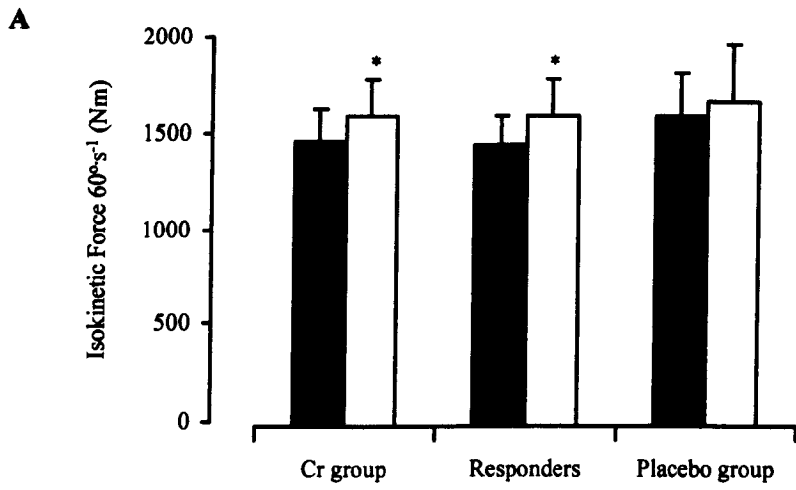
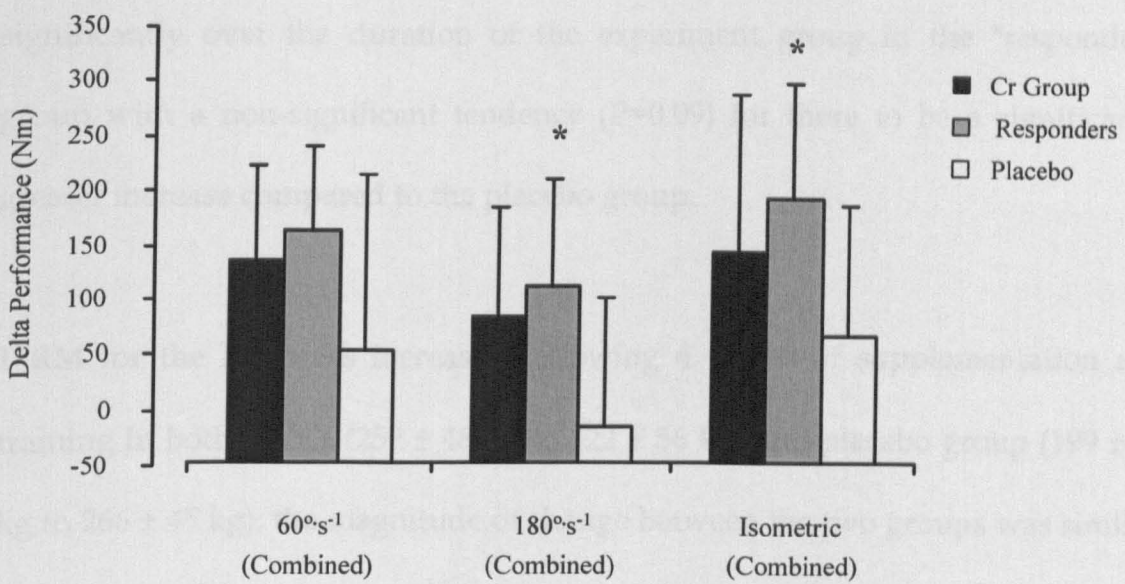


Figure 4.4

Change in isokinetic ($60^{\circ}\cdot\text{s}^{-1}$ and $180^{\circ}\cdot\text{s}^{-1}$) and isometric force in the Cr group, "responders" and placebo group (mean \pm s.d.). * indicates a significantly greater increase in the "responders" and/or Cr group compared to the placebo group



Training volume

Total lifting volume (calculated for every training session: weight lifted x repetitions x sets) increased significantly over the duration of the experiment in both groups, with no difference in the magnitude of the increase between groups (2149 ± 773 kg vs. 2578 ± 445 kg, $P=0.27$). Total lifting volume increased significantly over the duration of the experiment group in the "responders" group with a non-significant tendency ($P=0.09$) for there to be a significantly greater increase compared to the placebo group.

1 RM for the leg press increased following 4 weeks of supplementation and training in both the Cr (252 ± 48 kg to 322 ± 56 kg) and placebo group (199 ± 57 kg to 266 ± 45 kg); the magnitude of change between the two groups was similar. 1 RM increased significantly in the "responders" group following 4 weeks of supplementation and training (260 ± 42 kg to 327 ± 53 kg), with the magnitude of change not significantly different when compared to the placebo group ($P=0.94$).

Urinary Analysis

In the Cr group, Crea excretion increased from 1.5 ± 0.4 g·d⁻¹ at baseline to 3.3 ± 0.8 g·d⁻¹ on the final day of loading, while in placebo group, Crea excretion over the 4 weeks was not different from baseline (1.5 ± 0.4 g·d⁻¹ on baseline to 1.6

$\pm 0.3 \text{ g}\cdot\text{d}^{-1}$ on final day of loading). Daily Cr excretion was therefore corrected for this increase in Crea excretion in the Cr group, and Cr excretion increased compared to baseline at all time points; no urinary Cr was detected in the placebo group. The amount of Cr retained each day (i.e. only for days with urinary data) was calculated by subtracting the total Cr excreted (corrected for Crea excretion) from the total amount supplemented per day. The amount of Cr retained was $65 \pm 11\%$ ($13.0 \pm 2.1 \text{ g}$) of the supplemented dose (i.e. 20 g) on the first day of loading and decreased to $23 \pm 27\%$ ($4.6 \pm 5.4 \text{ g}$) on the final day of loading. Cr retention was $46 \pm 41\%$ ($2.3 \pm 2.0 \text{ g}$), $17 \pm 34\%$ ($0.9 \pm 1.7 \text{ g}$) and $58 \pm 32\%$ ($2.9 \pm 1.6 \text{ g}$) of the supplemented dose (i.e. 5 g) on days 14, 21 and 28, respectively. Estimated Cr uptake was calculated based on an estimated muscle mass of 40% of body mass and an average muscle water content of 77% of wet weight (Bergstrom *et al*, 1971). Estimated Cr uptake in the Cr group was $28.3 \pm 8.5 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{dry muscle weight}$. In the "responders" (n=7), estimated Cr uptake was 31.5 ± 6.3 or 28.8 (25.6–43.9) (median (range)) $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry muscle weight}$ compared to 18.7 and 14.8 $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry muscle weight}$ in the two subjects classed as "non-responders". Over the duration of the experiment, urine volume was not different between groups.

Correlations

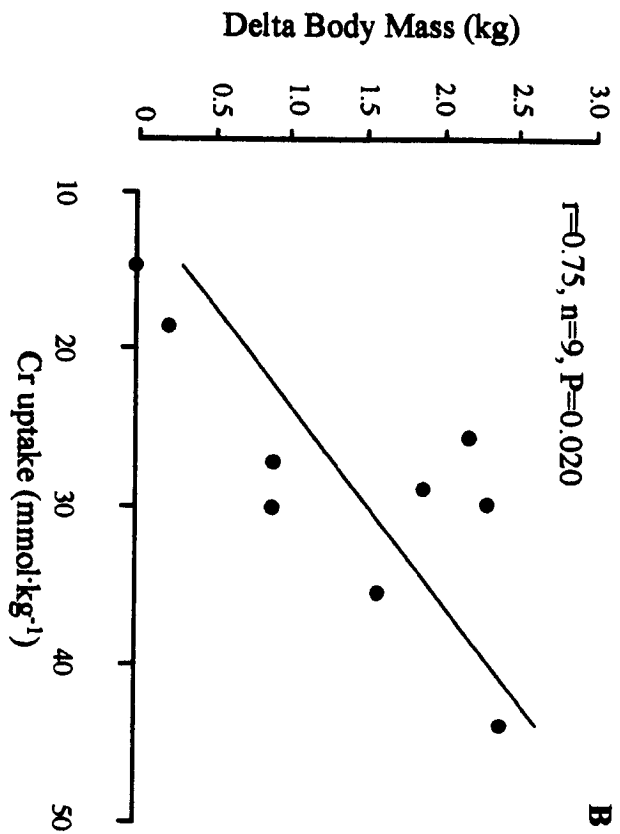
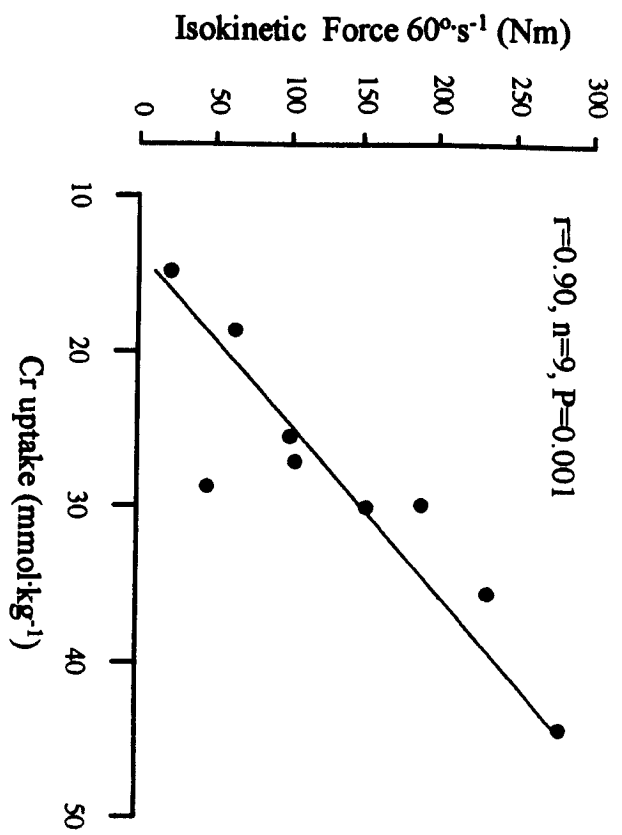
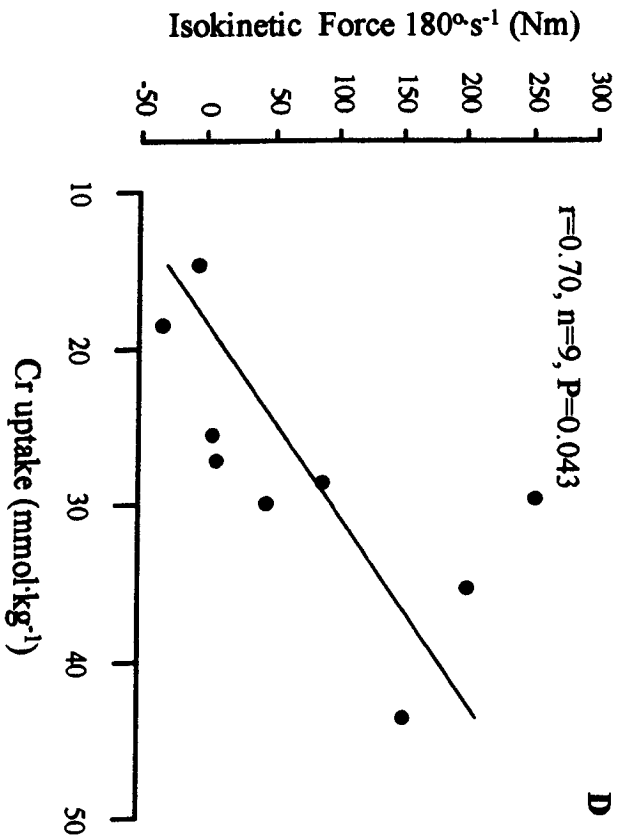
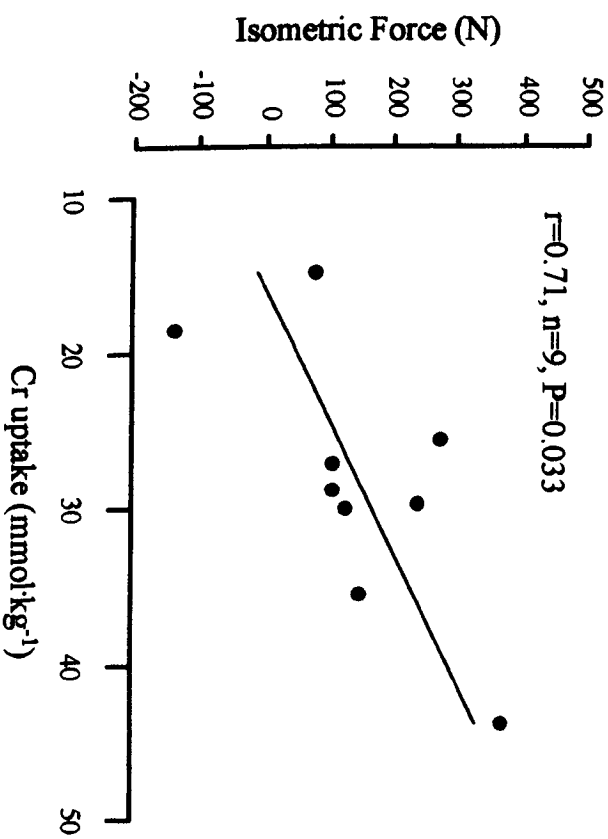
A significant positive correlation was found between change in body mass (loading phase) and change in $180^{\circ}\cdot\text{s}^{-1}$ isokinetic force ($r=0.68$, $n=9$, $P=0.04$) and isometric force ($r=0.82$, $n=9$, $P<0.01$), with a tendency for there to be a positive correlation with $60^{\circ}\cdot\text{s}^{-1}$ isokinetic force ($r=0.61$, $n=9$, $P=0.079$). The change in body mass over the maintenance phase was also correlated with $180^{\circ}\cdot\text{s}^{-1}$ isokinetic force ($r=0.70$, $n=9$, $P=0.037$). Estimated Cr uptake was positively correlated with the change in body mass (loading phase) ($r=0.75$, $n=9$, $P=0.02$) (Figure 4.5a), $60^{\circ}\cdot\text{s}^{-1}$ isokinetic force ($r=0.90$, $n=9$, $P=0.001$) (Figure 4.5b), $180^{\circ}\cdot\text{s}^{-1}$ isokinetic force ($r=0.68$, $n=9$, $P=0.043$) (Figure 4.5c) and isometric force ($r=0.71$, $n=9$, $P=0.033$) (Figure 4.5d). No significant correlations were found in the placebo group.

Side Effects/Treatment Identification

Subjects tolerated the supplementation protocol well, with no reports of gastrointestinal distress, muscle cramping or any other side effects. Subjects were asked at the end of the experiment whether they were aware of the treatment they had received and all but two subjects reported that they were unsure about the treatment they received (one subjects from each group identified correctly the treatment they were on).

Figure 4.5

Significant correlations between estimated Cr uptake and changes in body mass (A), isokinetic $60^{\circ}\cdot\text{s}^{-1}$ force (B), isokinetic $180^{\circ}\cdot\text{s}^{-1}$ force (C), and isometric force (D) in the Cr group. * indicates a significantly increase from pre to post

A**B****C****D**

DISCUSSION

The results of this experiment demonstrate that Cr supplementation is effective in increasing muscle strength in non-resistance trained subjects with significant Cr uptake during the supplementation period in conjunction with 4 weeks of resistance training compared to resistance training alone. Four weeks of Cr supplementation significantly increased isokinetic torque ($180^{\circ}\cdot\text{s}^{-1}$) and isometric force in a group of 7 "responders" compared with the placebo group (Figures 4.3 and 4.4). Without direct measurement of muscle [PCr], one can only speculate on the potential mechanisms for this improvement in muscle strength following Cr supplementation. Nevertheless, a number of previous studies suggest increased PCr availability and PCr resynthesis during recovery from maximal exercise as the most plausible (Balsom *et al*, 1995; Greenhaff *et al*, 1994). The results of the present Experiment are in agreement with a study by Maganaris & Maughan (1998); these authors found increases in MVC in subjects engaged in a weight-training program. These authors suggested that these increases could be a result of a Cr-stimulated increase in protein synthesis which, in turn, could lead to an increase in strength through muscle hypertrophy. However, the significance of such a mechanism during studies of this time scale (i.e. 5 days) remains unclear. Cr stimulated increase in protein synthesis could be a possible explanation for the increases in muscle strength observed in the present experiment due to the longer study duration (Kreider *et al*, 1998).

Despite many Cr supplementation studies showing an ergogenic effect on exercise performance, there are a significant number of studies which report no ergogenic effect (e.g. Cooke *et al*, 1995; Finn *et al*, 2001; McKenna *et al*, 1999; Snow *et al*, 1998). This is not surprising, however, as none of these studies differentiate subjects into "responders" and "non-responders" on the basis of measured or estimated muscle Cr uptake, or of changes in body mass. Had the subjects in the present experiment and in a previous experiment (Experiment 1) not been differentiated into "responders" and "non-responders", no ergogenic effect would have been detected (i.e. Type II error). However, classification of subjects into "responders" and "non-responders" should not simply be made on the basis of strength gains (as this would almost certainly increase the risk of producing a Type I error), but with good physiological justification. For example, there would be no obvious rationale for gains in strength if there was not a substantial increase in intramuscular [Cr] and, therefore the potential to increase PCr resynthesis following Cr supplementation. In the present experiment, subjects were differentiated into "responders" and "non-responders" on the basis of changes in body mass and not estimated muscle Cr uptake, as Cr uptake was calculated from urine samples collected only on days 1, 7, 14, 21 and 28 rather than from the entire 28 days. Nevertheless, estimated Cr uptake in the "responders" was 28.8 (25.6 - 43.9) mmol·kg⁻¹·dry muscle weight (median (range)) compared to 18.7 and 14.8 mmol·kg⁻¹·dry muscle weight in the two subjects classed as "non-responders" showing two distinct groups as previously reported

(Greenhaff *et al*, 1994; Experiment 1). This and the finding of a significant positive correlation between estimated Cr uptake and the change in body mass (Fig. 4.5a) is also in agreement with the results from Experiment 1 (Fig. 3.4c) and is further evidence supporting its use. Furthermore, a significant positive correlation was found between estimated Cr uptake and strength gains during 60°·s⁻¹ isokinetic force ($r=0.90$, $n=9$, $P=0.001$) (Figure 4.5b), 180°·s⁻¹ isokinetic force ($r= 0.68$, $n=9$, $P=0.043$) (Figure 4.5c) and isometric force ($r=0.71$, $n=9$, $P=0.033$) (Figure 4.5d), indicating that subjects with the greatest Cr uptake had the greatest strength gains as previously found by Casey *et al* (1996), and also found in Experiment 1 (Figure 3.4c).

As training status has the potential to influence Cr uptake, non resistance-trained subjects were used in the present Experiment. Despite this, two out of the nine subjects supplemented with Cr were clearly "non-responders" based on changes in body mass and urinary data. However, the ratio of "non-responders" to "responders" in the present subjects (2:9) was similar to the ratio previously reported in resistance-trained subjects (4:21) (Experiment 1). This finding would suggest that other factors, in addition to training status/experience, determine whether subjects will respond to Cr supplementation. One possible explanation for the two distinct groups (i.e. "responders" and "non-responders") may be the varying amount of intramuscular Cr prior to supplementation. For example, this may reflect a low habitual dietary intake of Cr in the "responders" and/or

conversely, a high dietary intake of Cr in the "non-responders". While subjects carried out a weighed intake of food, it was not possible to estimate accurately the dietary Cr content, as the amount of Cr in each food item is dependent on many factors including food preparation. Nevertheless, it would be reasonable to expect subjects with a high protein intake to also have a high Cr intake. However, no significant correlation between protein intake and estimated Cr uptake was found ($P = 0.51$).

A consistent finding throughout the Cr literature (e.g. Cooke *et al*, 1995; Finn *et al*, 2001; Ingwall *et al*, 1974) and in the present Experiment is a significant increase in body mass following both short and long term Cr supplementation. It has been suggested that the increase in body mass following Cr supplementation was due to an increase in water retention (Hultman *et al*, 1996), which could result in cell swelling, followed by an increase in protein synthesis (Haussinger *et al*, 1993). Others, however, have attributed the increase in body mass following Cr supplementation to an increase in protein synthesis and associated increase in water content (Kreider *et al*, 1998). Some of the justification for the increase in protein synthesis with Cr supplementation stems from the early work by Walker (1979), demonstrating that the amino acids glycine and arginine could stimulate protein synthesis. As dietary Cr consumption increases, endogenous production of Cr decreases, therefore allowing these amino acids to be conserved and therefore to be more freely available for protein synthesis (Walker, 1979). The

balance of available evidence from human performance studies using Cr supplementation and more direct evidence from animal *in vivo* and *in vitro* experiments would support the notion that increasing Cr availability may indeed increase protein synthesis (Ingwall *et al*, 1974; Kreider *et al*, 1998; Volek *et al*, 1999). Supplementation with Cr in the present Experiment increased body mass, with the mean increase in the responder group being greater than that of the placebo group after loading and maintenance (Figure 4.2a). This increase in body mass cannot be explained by the increase in TBW, reflecting Cr stimulated water retention, as there was no significant increase in TBW expressed as percentage of body mass. Despite a significant increase in TBW in absolute terms in the Cr group (Figure 4.2b), if the relative volume of TBW remains constant (as in the present experiment), the gain in body mass need not be attributed to water retention. Instead, the increase in absolute TBW seen after Cr supplementation may be indicative of intracellular water that normally accompanies dry matter growth. Similar results have previously been found and interpreted in the same manner (Francaux & Poortmans, 1999).

An additional component of the present Experiment was to evaluate the effects of Cr supplementation in conjunction with resistance-training on 1 RM and training volume performed on an isotonic leg press as compared to strength training alone. The physiological basis for a possible ergogenic effect of Cr supplementation on strength training was primarily two-fold. Firstly, Cr

supplementation has been shown to increase the number of repetitions performed per set (Earnest *et al*, 1995; Volek *et al*, 1997). Secondly, Cr supplementation has been shown to increase the rate of PCr rephosphorylation during the second minute of recovery from intense intermittent-type exercise (Greenhaff *et al*, 1994). Theoretically, both these physiological changes would allow an individual to train at a greater intensity compared to training without the use of this putative ergogenic aid. In the present experiment, however, Cr supplementation (Cr group and "responders" group) in conjunction with 4 weeks of resistance-training did not induce greater gains in 1 RM or total lifting volume when compared to the placebo group; subjects in the Cr, "responders" and placebo group increased their 1 RM on the leg press by 27 %, 27% and 34 %, respectively. There was however a tendency for the "responders" to have a greater total lifting volume compared to the placebo group ($P=0.09$); statistical significance may not have been reached due to the relatively small sample size ("responders" $=7$). This is in agreement with previous studies by Francaux and Poortmans (1999) and Bermon *et al* (1998). Francaux and Poortmans (1999) examined the effects of 6 weeks of resistance-training in conjunction with Cr supplementation on isokinetic force and found that Cr ingestion did not induce a greater increase in force, compared to resistance training alone; isokinetic force increased by about 6% after training in both the placebo and Cr groups. However, there was no measure or estimate of Cr uptake, and questions must be asked about their training stimulus (i.e. only 30% of MVC in session 1, increasing

progressively to approximately 43% of MVC in the final training session). Similarly, Bermon *et al* (1998) examined the effects of Cr supplementation in conjunction with 7 weeks of resistance training on strength and strength endurance in 32 elderly subjects. They also found that Cr supplementation did not provide any additional benefit to body composition and maximal dynamical strength, compared to resistance-training alone. The majority of other studies have, however, found Cr supplementation in conjunction with resistance training (of varying duration from 4 weeks to 12 weeks) to have an ergogenic effect on performance (Becque *et al*, 2000; Kelly *et al*, 1998; Kreider *et al*, 1998; Vandenberghe *et al*, 1997; Volek *et al*, 1999). However the majority of these studies using short-duration training (4 - 6 weeks) have used previously resistance-trained individuals (Becque *et al*, 2000; Kelly *et al*, 1998; Kreider *et al*, 1998). A likely explanation for the findings of the present experiment (i.e. no effect of Cr supplementation and 4 weeks of resistance training on 1 RM and training volume) is the experimental duration (4 weeks) and subject group used (non resistance trained individuals).

It is well established that the initial relatively large gains in muscle strength seen in individuals early on during resistance-training (as in the present Experiment) are mainly due to neural factors (Sale, 1988) such as increases in synchronisation of motor unit firing patterns, increased neural drive to the muscle, and inhibition of the protective mechanisms of the muscle (e.g. Golgi tendon organs). These

neural adaptations dominate early training gains, whereas, after approximately 8 weeks, most of the changes that occur are associated with muscle hypertrophy (Sale, 1988). Future studies should therefore differentiate subjects into "responders" and "non-responders" and use training durations of greater than 8 weeks in order to examine if Cr supplementation has any additive effect on training gains especially in non resistance-trained subjects. However, the present Experiment shows Cr supplementation is effective in increasing muscle strength in this subject group during this time scale.

CONCLUSION

The results of this Experiment indicate that Cr supplementation in combination with strength training is effective in increasing muscle strength (as measured by isokinetic and isometric tests) but not 1 RM or training volumes in subjects whose intramuscular [Cr] and body mass are significantly increased; the greater the Cr uptake and associated body mass changes, the greater the performance gains. Cr supplementation studies should therefore differentiate subjects into "responders" and "non-responders" on the basis of measured or estimated Cr uptake and/or changes in body mass when assessing the effects on performance.

CHAPTER FIVE

(Experiment 3)

Effects of Creatine Supplementation on Thermoregulation and Exercise Performance in the Heat in Endurance- trained Humans

INTRODUCTION

The process of fatigue varies considerably with the mode, intensity and duration of exercise, as well as with environmental conditions. Fatigue in conditions ranging from short-duration, high intensity exercise to more prolonged endurance-type exercise performed in normal ambient temperatures, has been extensively studied and is well characterised. However, the mechanisms underlying fatigue during prolonged exercise in the heat remains uncertain, with theories ranging from the attainment of a critical core temperature (Nielsen *et al*, 1993) to evidence of serotonergic system involvement in the fatigue process (e.g. Pitsiladis *et al*, 2002). Others have suggested that fatigue during exercise in the heat is unlikely to be the result of glycogen depletion, and have attributed fatigue to factors such as hypohydration and/or some failure of the thermoregulatory system (e.g. Pitsiladis & Maughan, 1999).

Irrespective of the cause of fatigue, temperature regulation and performance during exercise in the heat are critically dependent on hydration status (Sawka & Pandolf, 1990). Numerous strategies have been designed to improve exercise performance by minimising the detrimental effects of dehydration on metabolism and thermoregulation during exercise in the heat. Such strategies include pre-cooling (Olschewski & Bruck, 1988; Lee & Haymes, 1995), fluid ingestion (Galloway & Maughan, 2000), and plasma volume expansion (Watt *et*

al, 2000). The effectiveness of some of these strategies during exercise in the heat remains to be determined.

Creatine supplementation has been widely used to improve performance during high-intensity, short duration exercise. Increasing intramuscular [TCr] by oral Cr supplementation (Harris *et al*, 1992) has been shown to increase intramuscular phosphocreatine levels and accelerates the PCr resynthesis rate following high-intensity, short-duration exercise (Greenhaff *et al*, 1994). There is evidence from Cr supplementation studies that oral Cr loading can increase muscle Cr content (Harris *et al*, 1992), improve anaerobic exercise performance (Casey *et al*, 1996; Green *et al*, 1996) and promote greater gains in strength (Kreider *et al*, 1998; Maganaris & Maughan, 1998). A Cr-stimulated increase in body mass has been consistently reported following Cr supplementation, but the precise mechanism of this remains unclear. The increase in body mass following Cr supplementation may be due to water retention (Hultman *et al*, 1996), cell swelling and a consequent increase in protein synthesis (Haussinger *et al*, 1993). Others have, however, suggested the reverse, i.e., an increase in protein synthesis and associated increase in water content (Kreider *et al*, 1998). Despite the uncertainty of the primary and secondary effects, Cr supplementation has consistently been shown to increase total body water (TBW), and more specifically, intracellular water (ICW) (e.g. Francaux & Poortmans, 1999; Experiment 2). Thus, it seems logical that Cr supplementation and the resulting cellular hydration may be beneficial in prolonging exercise in the heat.

Based, on the potential of Cr to increase TBW and ICW, the aim of this study was therefore to examine the effects of a Cr-induced hyperhydration on cardiovascular, metabolic, and thermoregulatory responses, and on the capacity to perform prolonged exercise in the heat.

METHODS

Subjects

Twenty one endurance-trained male volunteers had written informed consent obtained (Table 5.1). The study was approved by the local ethics committee. Subjects were recruited from local athletics and cycling clubs, and none were acclimatised to exercise in the heat. Subject eligibility was initially assessed by interview. No subject had a history of cardiovascular or respiratory disease and/or evidence of musculoskeletal injury. All subjects were Cr-free for at least 8 weeks prior to the study. The investigators did not reveal prior to interview that subjects would be excluded if they had supplemented with Cr in the 8 weeks preceding the study. One subject from the placebo group had previously supplemented with Cr. No Cr was detected in the baseline urine samples of any subject.

Experimental Design

Subjects initially underwent a continuous incremental test to volitional exhaustion in order to determine the lactate threshold (LT), $\dot{V}O_2$ max and WR max. LT was estimated non-invasively as the $\dot{V}O_2$ at which: (a) the break-point in the relationship between CO_2 output ($\dot{V}CO_2$) and $\dot{V}O_2$ ("V-slope" technique, Beaver *et al*, 1986) occurred and (b) the ventilatory equivalent for O_2 ($\dot{V}E / \dot{V}O_2$)

Table 5.1: Physical characteristics of the two groups of subjects

	Placebo Group (n=10)		Creatine Group (n=11)	
	Pre	Post	Pre	Post
Age (yr)	27 ± 4	-	27 ± 5	-
Height (m)	1.81 ± 0.04	-	1.78 ± 0.07	-
Body mass (kg)	71.0 ± 6.0	71.2 ± 6.0	72.7 ± 6.6	73.4 ± 6.6*
Total body water (L)	40.4 ± 3.3	40.5 ± 3.2	41.0 ± 3.1	41.6 ± 3.2*
Intracellular water (L)	21.9 ± 1.8	21.9 ± 1.8	22.2 ± 1.8	22.7 ± 1.9*
Extracellular water (L)	18.5 ± 1.5	18.5 ± 1.4	18.8 ± 1.4	18.9 ± 1.5
$\dot{V}O_2$ max (L/min)	4.3 ± 0.4	-	4.5 ± 0.4	-
$\dot{V}O_2$ max (ml · kg ⁻¹ · min ⁻¹)	60.5 ± 4.7	-	61.4 ± 4.6	-
Work rate _{max} (W)	350 ± 34	-	373 ± 31	-

Values are presented as the mean ± s.d.

* Indicates a significant difference from pre-supplementation values

started to increase systematically without a concomitant increase in the ventilatory equivalent for CO₂ ($\dot{V}_E / \dot{V}_{CO_2}$) (Whipp *et al*, 1986).

An electrically-braked cycle ergometer (Bosch Erg-551 Forckenbecksti, Berlin, Germany), was set at an incrementation rate of 20 W min⁻¹ (starting at 20 W). Expired gases were collected for one minute during each incremental stage using Douglas bags and analysed within 5 min of collection for mixed expired [O₂] (Servomex 570A, East Sussex, UK), mixed expired [CO₂] (Servomex 1400 B4, East Sussex, UK), gas volume (dry gas meter, Harvard Apparatus Ltd., Hertfordshire, UK), and expired gas temperature (C6600 10-channel microprocessor, Comark, Hertfordshire, UK). The gas analysis system was calibrated before each test, with the CO₂ and O₂ analyzers calibrated using a two-point measure: a calibration gas (CO₂ 7.5%, O₂ 16%, N₂ balance, certified standard gas) and a reference gas (room air). Barometric pressure was measured using a standard mercury barometer.

Following the maximal incremental exercise test, subjects visited the laboratory on at least two occasions in order to become familiar with the exercise protocol and experimental procedures, in addition to establishing a suitable work rate that would elicit fatigue in 40 - 60 minutes. This was achieved by setting the work rate at 20% Δ (i.e. 20% of the difference between the $\dot{V}O_2$ at the LT and $\dot{V}O_2$ max which is subsequently added back on to the $\dot{V}O_2$ at the LT) during the initial familiarisation session and, where necessary, adjusting the work rate for

subsequent trials to achieve the desired duration. This intensity of exercise was chosen to avoid fatigue occurring as a result of muscle glycogen depletion. Following the familiarisation period, all subjects performed two constant-load exercise tests to volitional exhaustion pre- and post-supplementation. The first test was conducted not less than 48 hrs after the subject's final familiarisation trial. The supplementation period for both groups started on the day after the first test and finished the day before the second test.

Cr supplementation consisted of $22.8 \text{ g}\cdot\text{d}^{-1} \text{ Cr}\cdot\text{H}_2\text{O}$ (equivalent to $5 \text{ g Cr} \times 4$ daily) and 35 g of glucose polymer made up in 500 mls of warm to hot water for 7 days taken at equal intervals throughout the day. This protocol has been shown to increase resting muscle PCr levels within 5 days (Harris *et al*, 1992). The addition of dextrose to Cr significantly enhances the uptake of Cr (Green *et al*, 1996). The placebo group consumed $160 \text{ g}\cdot\text{d}^{-1}$ of glucose polymer ($40 \text{ g} \times 4$ daily) for 7 days, prepared and administered in an identical fashion to the Cr supplement. Both supplements had similar taste, texture and appearance and were placed in generic packets to ensure double-blind administration.

Subjects otherwise followed their normal diet and weighed all food and drink consumed during the supplementation period using digital weighing scales readable to 1 g . The diet was analysed for energy intake and macronutrient content (Holland *et al*, 1991). Subjects eliminated caffeine and caffeine-containing foods from their diet to minimise the possible inhibitory effects of caffeine on the ergogenic effect of Cr (Vandenberghe *et al*, 1996). Subjects

maintained their normal training habits for the duration of the study. At the end of the study all subjects gave verbal assurance that they had complied with these instructions. Subjects completed 8 separate 24 hr urine collections. The collection began on the day preceding supplementation (baseline), then continued through the 7 days of supplementation. The urine volume for each 24 hr period was measured and mixed thoroughly, with a representative 20 mL sample being stored at -20 °C for subsequent analysis (ABX Mira Plus Spectrophotometer, ABX Diagnostics, UK) of [Cr] and [creatinine] ([Crea]) using a spectrophotometric enzymatic Crea Kit (MPR1 - Kit no. 839434, Roche Diagnostics Ltd., East Sussex, UK). Estimated Cr uptake was calculated by subtracting the total Cr excreted, corrected for Crea excretion, from the total amount supplemented per day. Estimated intramuscular [Cr] ($\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$) was calculated based on an estimated muscle mass amounting to 40% of body mass and average muscle water approximating 77% of wet weight (Bergstrom *et al*, 1971).

Procedures

All exercise tests were carried out between 16:00 and 20:00 hr. Subjects reported to the laboratory on the day of testing after a standardised meal and having refrained from alcohol, caffeine and strenuous exercise the day before. Height and nude body mass were measured, and TBW, ICW and ECW estimated using a standard bioimpedance technique (Bodystat-5000 Bioimpedance analyser, Bodystat Ltd., Isle of Man) (Van Loan *et al*, 1990). A

flexible rectal thermistor was inserted 10 cm past the anal sphincter to measure rectal temperature (T_{rec}), an index of core temperature. A heart rate (HR) monitor (Polar Sports Tester, Polar Electro Oy, Kempele, Finland) was positioned, and thermistors (C6600 10-channel microprocessor, Comark, Hertfordshire, UK) attached to the chest, upper arm, thigh and calf for the determination of weighted mean skin temperature (T_{skin}) (Ramanathan, 1964). A 5 ml arterialised-venous (Forster *et al*, 1972) resting blood sample (heating lamp) was obtained from a superficial vein on the dorsal surface of the hand. The subject was transferred to the climatic chamber (ambient temperature of 30.3 ± 0.5 °C with a relative humidity of $70 \pm 2\%$ and air velocity of approximately $3.6 \text{ m}\cdot\text{s}^{-1}$) and remained seated on the cycle ergometer for a further 5 min while resting HR, T_{rec} , T_{skin} and gas collections were obtained. Subjects were then instructed to begin 5 min of unloaded cycling before further measurements and another blood sample were obtained. After 5 min of unloaded cycling, the work rate was increased in a "single step" to the predetermined power output and subjects maintained a pedal cadence of 60 - 90 rpm throughout the test. Subjects exercised at $16 \pm 11\% \Delta$ or $63 \pm 5\% \dot{V}O_2 \text{ max}$. Exhaustion was defined as the point at which the subject could no longer maintain the pedal cadence above 60 rpm. Blood samples and measurements of HR, T_{rec} and T_{skin} were obtained at 5 min intervals throughout exercise and at exhaustion. One minute expired gas collections were made every 5 min and analysed within 5 min for the determination of $\dot{V}O_2$, $\dot{V}CO_2$ and respiratory exchange ratio (RER). Subjective ratings of perceived leg tiredness and breathlessness were recorded every 5 min

until exhaustion using the Borg category scale (Borg, 1982). After exercise, nude body mass was measured. The difference in body mass before and after exercise was calculated and subsequently used to estimate sweat rate and sweat loss after correcting for respiratory water loss and substrate oxidation (Mitchell *et al*, 1972). Time to exhaustion was recorded but withheld from the subject until all exercise tests had been completed.

Blood Treatment and Analysis

Blood was drawn into dry syringes and 5 mL dispensed into a tube containing K₃EDTA. Duplicate aliquots (400 µL) of whole blood from the K₃EDTA tube were rapidly deproteinised in 800 µL of ice cold 0.3 mol·L⁻¹ perchloric acid, centrifuged and the supernatant was used for the measurement of glucose and lactate (Maughan, 1982). Blood from the K₃EDTA tube was analysed for haemoglobin (Hb) (cyanmethaemoglobin method, Sigma Chemical Company Ltd., Dorset, UK) and packed cell volume (PCV) (conventional microhematocrit (Hct) method). All blood analyses were carried out in duplicate with the exception of PCV, which was analysed in triplicate. Plasma volume changes were calculated from changes in Hb and PCV relative to initial baseline values (Dill & Costill, 1974).

Calculations

Weighted mean skin temperature [$T_{\text{skin}}=0.3(T_{\text{chest}}+T_{\text{arm}}) +0.2(T_{\text{thigh}}+T_{\text{calf}})$] (Ramanathan, 1964) and mean body temperature (T_b) [$0.87T_{\text{rec}}+0.13T_{\text{skin}}$] (Olschewski & Bruck, 1988) were calculated for each time point. Metabolic rate was calculated for each time point using the following equation: metabolic rate = $[4.686+(RQ-0.707/0.293)0.361] \dot{V}O_2$ (Ravussin *et al*, 1985). Mechanical efficiency ($ME=(WR/69.67)/[\dot{V}O_2, ((1.1891RQ)+3.851)]100$), and net mechanical efficiency ($NME=(WR/69.67)/[(\dot{V}O_2-(BM0.004))((1.1891RQ)+3.851)]100$) were also calculated.

Data Analysis

Data were expressed as the mean \pm s.d. or median (range), following a test for the normality of distribution. Subjects in the Cr group were classified as "responders" and "non-responders" based on estimated Cr uptake (Greenhaff *et al*, 1994; Casey *et al*, 1996; Experiment 1; Experiment 2), and both Cr group as a whole and "responders" were compared to the placebo group. Statistical analysis was carried out using two factor ANOVA for repeated measures, followed by students paired *t*-test (within treatment effect, i.e. pre- vs. post-supplementation) and two-sample *t*-test (between treatment effect, i.e. magnitude of change (Δ) in the Cr group or "responders" vs. Δ in the placebo group) if a main treatment or interaction effect was observed. An ANCOVA

was used where necessary to normalise for differences in pre-supplementation results using the baseline value as the covariate. Pearson's correlation analysis was used to assess the relationship between selected variables. Statistical significance was declared at $P \leq 0.05$.

RESULTS

Estimated Cr Uptake

In the Cr group, Crea excretion increased from $1.4 \pm 0.4 \text{ g}\cdot\text{d}^{-1}$ pre-supplementation to $2.4 \pm 1.0 \text{ g}\cdot\text{d}^{-1}$ on the final day of supplementation. There was no increase in Crea excretion in the placebo group ($1.5 \pm 0.4 \text{ g}\cdot\text{d}^{-1}$ to $1.4 \pm 0.4 \text{ g}\cdot\text{d}^{-1}$). Cr excretion increased from $8.7 \pm 3.7 \text{ g}\cdot\text{d}^{-1}$ pre-supplementation to $17.4 \pm 1.9 \text{ g}\cdot\text{d}^{-1}$; no Cr was detected in the urine of the placebo group. Estimated Cr uptake was maximal on the first day of Cr supplementation (12 (6 - 15) g, 61 (32 - 77)% being retained) and was lowest on the final day (3 (-2 - 4) g, 16 (-8 - 21)% being retained). The total amount of Cr retained over the supplementation period was $39 \pm 14 \text{ g}$, with an estimated increase in intramuscular [Cr] of 51 (21 - 61) $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$. Based on these estimates, 3 subjects were classified as "non-responders" (21 (21 - 25) $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$) and the remaining 8 subjects were classified as "responders" ($53 \pm 5 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{dry weight muscle}$).

Time to Exhaustion

Time to exhaustion (TTE) was not significantly different between the groups prior to supplementation ($P=0.27$). Pre- compared to post-supplementation, TTE were not significantly different in either the placebo ($50.4 \pm 8.4 \text{ min}$ to $51.2 \pm 8.0 \text{ min}$, $P=0.119$, 95 % C.I. 1.8 to -0.3) nor Cr ($47.0 \pm 4.7 \text{ min}$

to 49.7 ± 7.5 min, $P=0.095$, 95% C.I. 6.0 to -0.6) group; Δ exercise performance was also not different between groups ($P=0.24$). TTE was significantly increased following supplementation in the "responders" (47.3 ± 4.9 min to 51.7 ± 7.4 min, $P=0.031$, 95% C.I. 8.3 to 0.5), with a tendency ($P=0.066$) for Δ exercise performance to be greater in the "responders" (Figure 5.1).

Diet, Body Mass and Body Water Compartments

The physical characteristics of the two groups of subjects were similar before supplementation (Table 5.1). In the Cr group, body mass increased significantly following supplementation, with no change in the placebo group (Δ body mass was greater in the Cr group, Figure 5.2). There was no difference pre-supplementation in TBW, ICW and ECW between groups (Table 5.1). In the Cr group, TBW and ICW increased significantly following supplementation. TBW and ICW were unaltered by supplementation in the placebo group (Δ TBW and ICW were greater in the Cr group, Figure 5.2). There was no significant increase in ECW in either group following supplementation (Figure 5.2). Following supplementation in the "responders", there was a significant increase in body mass (72.7 ± 7.8 kg to 73.5 ± 7.8 kg) (Δ body mass was greater in the "responders" compared to the placebo group). In the "responders", TBW and ICW increased significantly from 40.9 ± 3.5 L to 41.7 ± 3.7 L and 22.1 ± 2.0 L to 22.7 ± 2.1 L, respectively following supplementation (Δ TBW and ICW were greater in the "responders"). There were no significant differences in the daily

Figure 5.1

Time to exhaustion (mean \pm s.d.) in the Cr, "responders" and placebo supplemented groups. * indicates a significant difference between pre- and post-supplementation

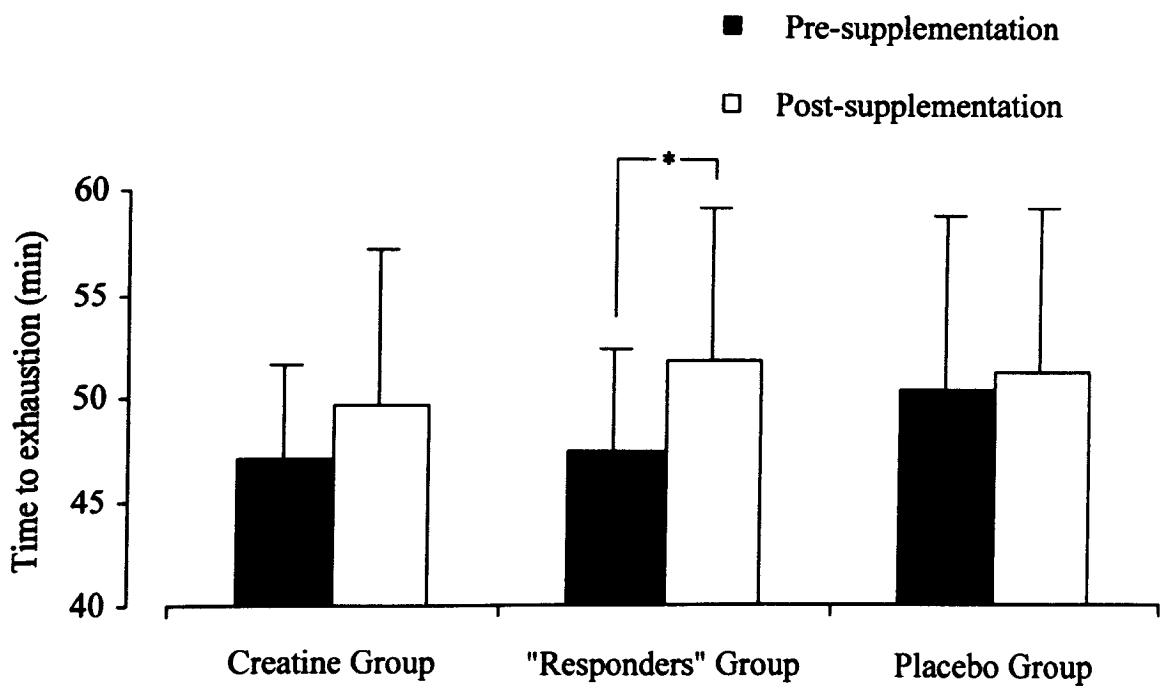
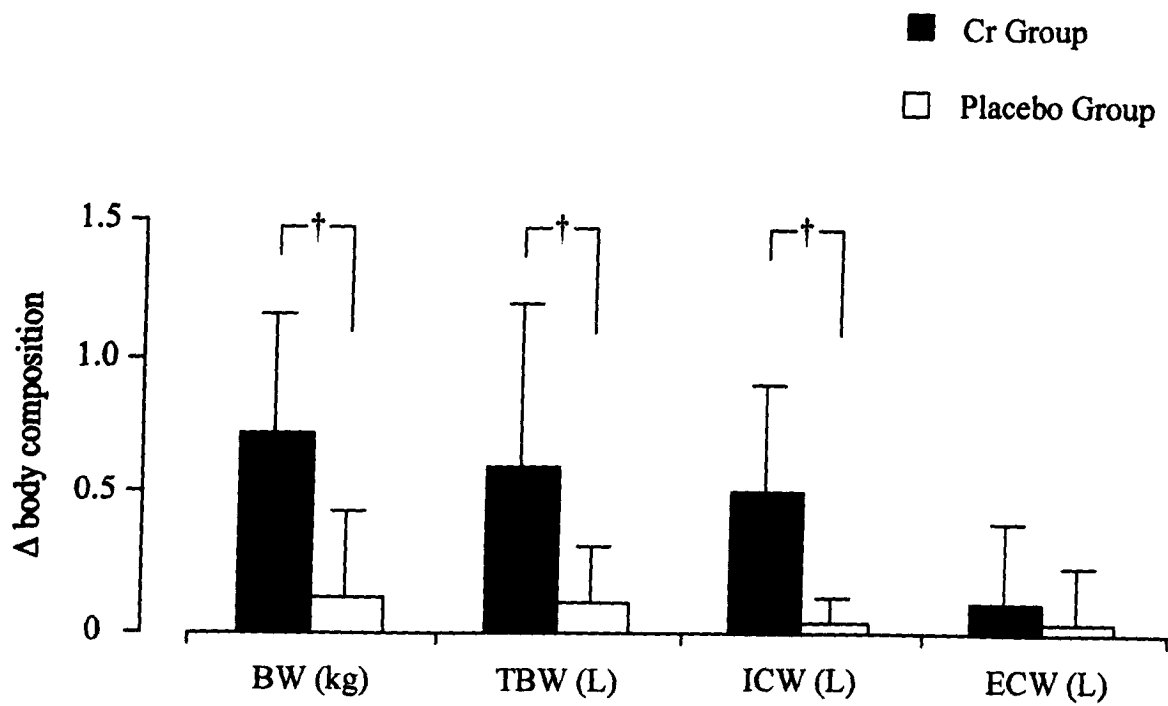


Figure 5.2

Changes in body weight (BW), total body water (TBW), intracellular water (ICW) and extracellular water (ECW) (mean \pm s.d.). † indicates a significant greater change in the Cr group compared to the placebo group



diet between the two groups (Cr: 14.0 ± 1.6 MJ·d⁻¹, $66 \pm 5\%$ carbohydrate, $21 \pm 6\%$ fat, $13 \pm 2\%$ protein; Placebo: 12.7 ± 2.3 MJ·d⁻¹, $63 \pm 3\%$ carbohydrate, $23 \pm 3\%$ fat, $14 \pm 2\%$ protein).

Work Efficiency during Exercise.

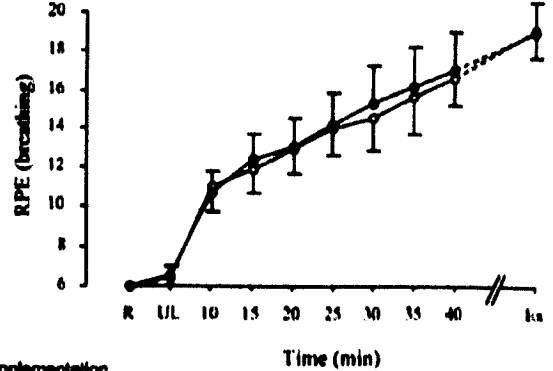
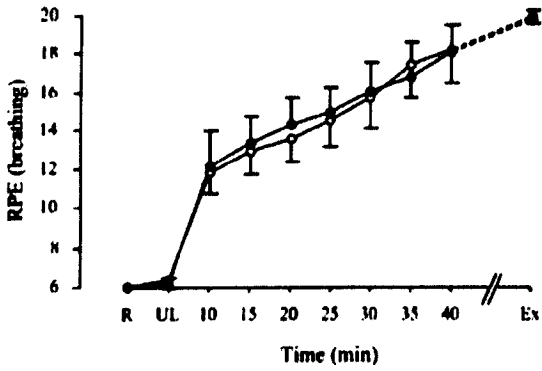
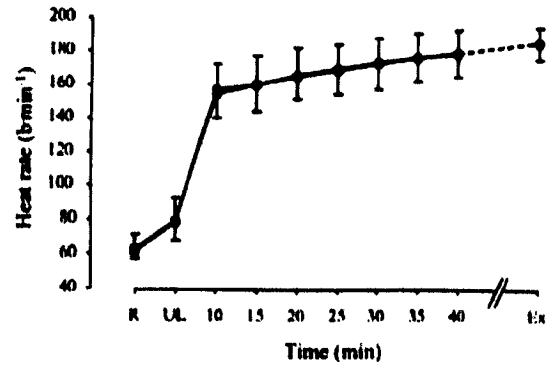
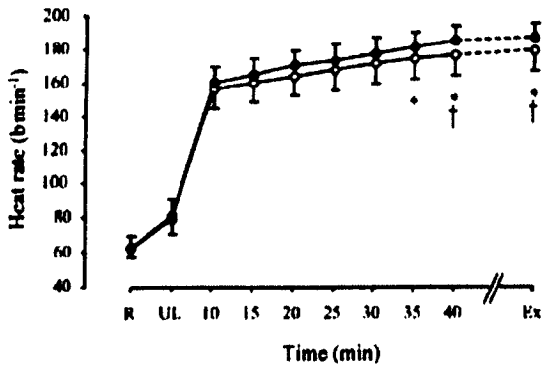
Mechanical and net mechanical efficiency increased in all groups during exercise and no difference was found between groups or following supplementation. Mechanical efficiency increased from approximately 9% at unloaded exercise to 20% at the end of exercise, and net mechanical efficiency increased from approximately 13% at unloaded exercise to approximately 22% at the end of exercise on all trials.

Heart Rate and Rating of Perceived Exertion during Exercise.

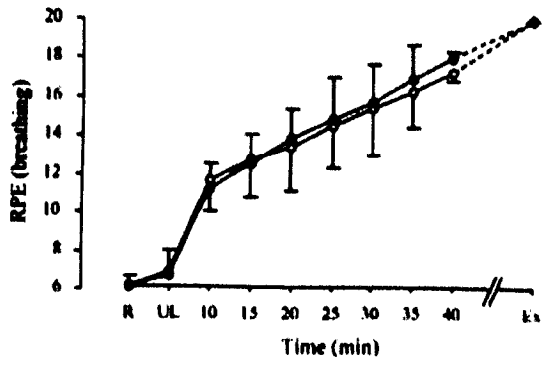
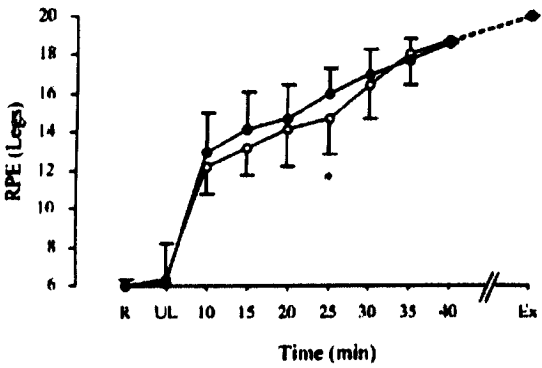
There was no difference in resting heart rate between the two groups of subjects before or after supplementation (Figure 5.3). During exercise, there was a uniform increase in HR in both placebo trials (Figure 5.3). In the Cr group, HR during exercise following supplementation was significantly lower from 35 min of exercise until exhaustion compared to pre-supplementation (Figure 5.3) (Δ HR was greater in the Cr group at 40 min of exercise and at exhaustion). There was no significant difference in exercising heart rate between "responders" and the placebo group. A progressive increase in RPE both for breathlessness and perceived leg fatigue was found during exercise

Figure 5.3

Heart rate (top panel), RPE (breathing) (middle panel) and RPE (legs) (bottom panel) in the Cr (left side) and placebo (right side) supplemented groups during exercise. * indicates a significant difference between pre to post supplementation. † indicates a significant greater change in the Cr group compared to the placebo group.



● Pre-supplementation
○ Post-supplementation



reaching near maximum ratings at exhaustion (Figure 5.3). Both in the Cr group as a whole and in the "responders", significantly lower ratings of perceived leg fatigue were found after 25 min of exercise ($P=0.01$ and $P=0.008$, respectively), with tendencies at the 10 min (Cr group, $P=0.068$) and 15 min (Cr group, $P=0.076$; "responders", $P=0.097$); no such effect was found in the placebo group. There was also a tendency for less breathlessness in the Cr group ($P=0.067$). Five out of the eleven subjects in the Cr group reported that they found the post-supplementation trial easier, while two out of the ten subjects in the placebo group rated the post-supplementation trial to be easier. All other subjects rated both trials similarly (including the 3 "non-responders" to Cr supplementation).

Metabolic Rate and Body Temperature Response

Metabolic rate did not differ in the placebo group pre- and post-supplementation (Table 5.2). In the Cr group a significantly lower metabolic rate was found post-supplementation at 20 min, 25 min, 30 min and 40 min (Table 5.2) (Δ metabolic rate tended ($P=0.096$) to be greater in the Cr group). In the "responders", metabolic rate was significantly lower post-supplementation from 20 min to 40 min inclusive (Δ metabolic rate tended ($P=0.094$) to be greater in the "responders"). Rectal (T_{rec}), mean skin (T_{skin}) and mean body (T_{b}) temperature responses are shown in Figure 5.4. In the placebo group, all three body temperature measurements increased during exercise with no significant differences between trials. In contrast, in the Cr group, T_{rec} was lower at 35 min,

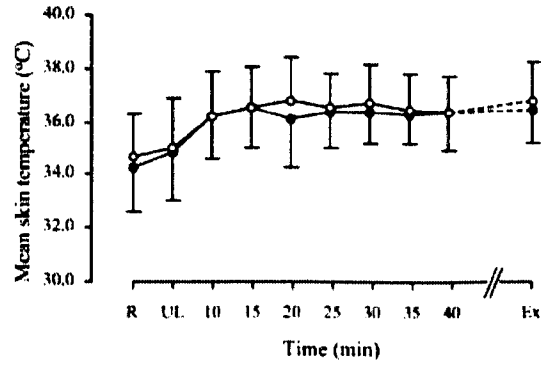
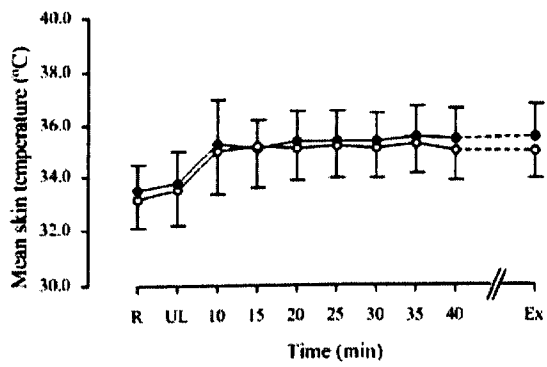
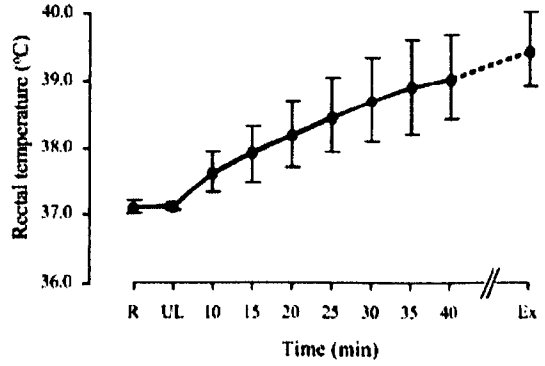
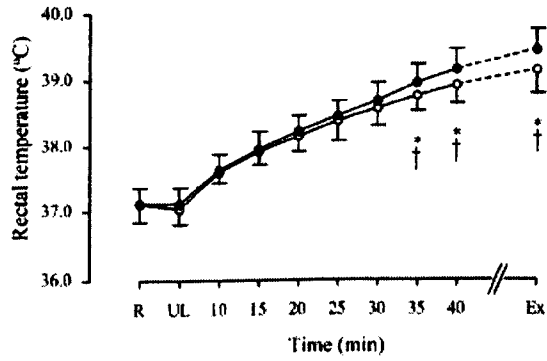
Table 2 Cardiopulmonary responses of the two groups of subjects before and after supplementation

Group	Trial	Exercise Time (min)										
		Rest	Unloaded	10	15	20	25	30	35	40		
Metabolic Rate (W·m ⁻²)	Cr	Pre	27 ± 4	55 ± 10	227 ± 19	230 ± 19	244 ± 13	245 ± 16	249 ± 15	251 ± 18	257 ± 24	
		Post	27 ± 4	49 ± 10	224 ± 16	231 ± 13	233 ± 10*	234 ± 13*	236 ± 12*	236 ± 22*	242 ± 18*	
	Plac	Pre	24 ± 7	48 ± 12	223 ± 31	224 ± 32	231 ± 28	226 ± 26	234 ± 26	234 ± 26	227 ± 37	237 ± 33
		Post	27 ± 3	55 ± 13	223 ± 22	227 ± 27	230 ± 26	234 ± 26	233 ± 24	237 ± 26	229 ± 25	
	VO ₂ (L/min)	Cr	Pre	0.4 ± 0.1	0.8 ± 0.1	3.0 ± 0.4	3.3 ± 0.4	3.2 ± 0.3	3.2 ± 0.4	3.3 ± 0.4	3.3 ± 0.4	3.4 ± 0.5
		Post	0.4 ± 0.1	0.7 ± 0.1	3.0 ± 0.2	3.1 ± 0.2	3.2 ± 0.2	3.2 ± 0.3	3.2 ± 0.3	3.2 ± 0.3	3.2 ± 0.3	3.3 ± 0.3
VCO ₂ (L/min)	Cr	Pre	0.3 ± 0.1	0.7 ± 0.2	3.0 ± 0.2	3.2 ± 0.5	3.2 ± 0.3	3.3 ± 0.3	3.3 ± 0.3	3.3 ± 0.3	3.4 ± 0.4	
	Post	0.3 ± 0.1	0.6 ± 0.2	3.0 ± 0.2	3.0 ± 0.2	3.1 ± 0.2*	3.1 ± 0.2*	3.1 ± 0.2*	3.1 ± 0.3	3.1 ± 0.3	3.2 ± 0.3*	
VE (L/min)	Cr	Pre	10.4 ± 3.5	17.8 ± 4.0	63.3 ± 9.6	71.0 ± 12.1	71.2 ± 9.3	74.0 ± 9.6	77.5 ± 9.9	81.0 ± 12.3	88.6 ± 17.9	
	Post	9.6 ± 2.3	16.4 ± 5.0	63.9 ± 8.2	68.9 ± 8.5	68.9 ± 8.2	71.5 ± 8.1	75.1 ± 9.0	78.1 ± 13.8	82.5 ± 14.1		
RER	Cr	Pre	0.88 ± 0.18	0.89 ± 0.14	1.03 ± 0.12	0.99 ± 0.15	1.02 ± 0.11	1.01 ± 0.10	1.00 ± 0.11	1.00 ± 0.09	1.00 ± 0.10	
	Post	0.88 ± 0.15	0.83 ± 0.16	0.98 ± 0.07	0.97 ± 0.06	0.97 ± 0.07	0.97 ± 0.06	0.97 ± 0.06	0.97 ± 0.05	0.97 ± 0.05	0.99 ± 0.06	
	Plac	Pre	0.85 ± 0.12	0.86 ± 0.14	0.97 ± 0.07	0.96 ± 0.05	0.96 ± 0.05	0.96 ± 0.05	0.97 ± 0.05	0.96 ± 0.06	0.95 ± 0.05	
	Post	0.86 ± 0.09	0.90 ± 0.17	0.97 ± 0.06	0.96 ± 0.05	0.97 ± 0.05	0.96 ± 0.06	0.96 ± 0.06	0.96 ± 0.06	0.95 ± 0.05	0.95 ± 0.05	

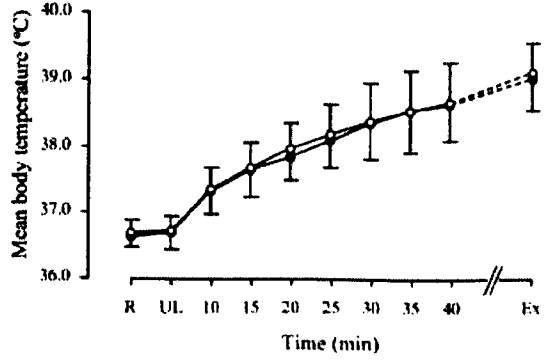
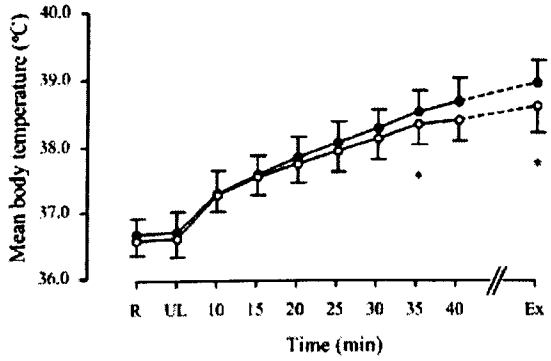
* Indicates a significant difference from pre-supplementation values

Figure 5.4

Rectal temperature (top panel), mean skin temperature (middle panel) and mean body temperature (bottom panel) in the Cr (left side) and placebo (right side) supplemented groups. * indicates a significant difference between pre to post supplementation. † indicates a significant greater change in the Cr group compared to the placebo group



● Pre-supplementation
○ Post-supplementation



40 min and at exhaustion following supplementation compared to pre-supplementation (ΔT_{rec} was greater in the Cr group from 35 min of exercise until exhaustion). Similarly, in the "responders", T_{rec} was lower at 40 min of exercise and at exhaustion following supplementation compared to pre-supplementation (ΔT_{rec} was greater in the "responders" at 40 min of exercise and at exhaustion).

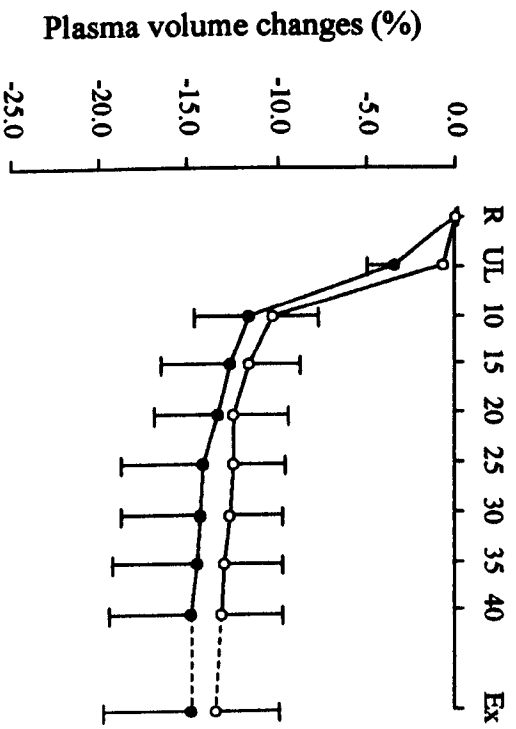
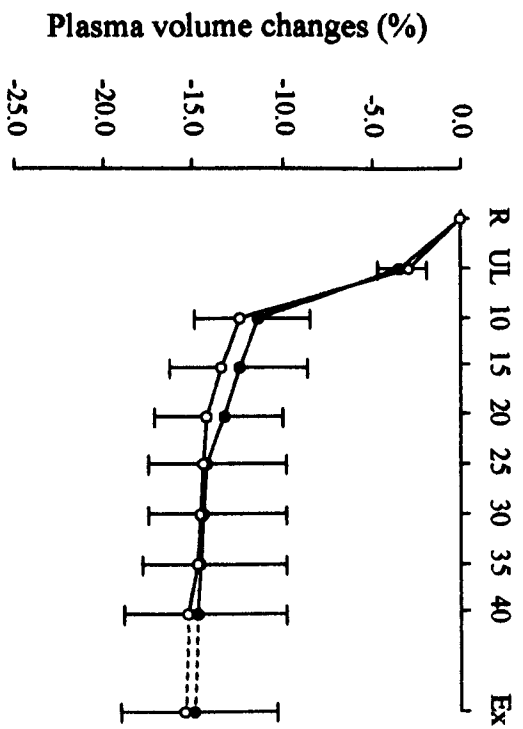
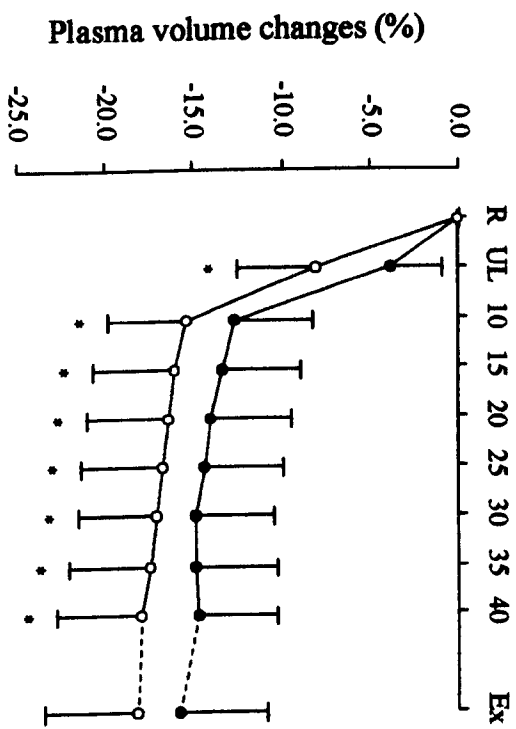
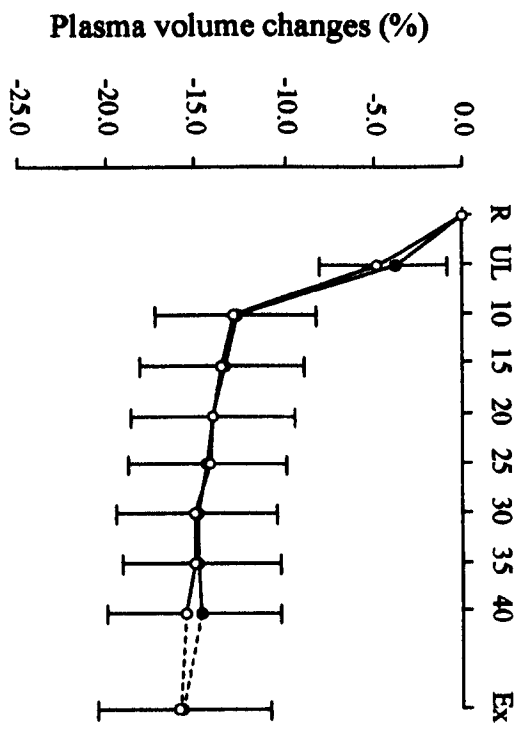
In the Cr group, a significantly lower T_b was found following supplementation at 35 min of exercise and at exhaustion, with tendencies for lower T_b at 30 min ($P=0.065$) and 40 min ($P=0.056$) (ΔT_b was greater in the Cr group at 20 min, 25 min, 30 min, 35 min and at exhaustion). In the "responders", a significantly lower T_b was found following supplementation at exhaustion ($P=0.014$) (ΔT_b tended ($P=0.083$) to be greater in the "responders"). There was a significant increase in mean T_{skin} with no significant differences between groups or following supplementation.

Sweat Rates and Total Sweat Loss during Exercise

There was a significant reduction in sweat rate following Cr supplementation (32.3 ± 7.0 ml min⁻¹ vs. 28.2 ± 3.9 ml min⁻¹; $P=0.02$) (Figure 5.6), no such reduction was observed in the placebo group (27.1 ± 9.8 ml min⁻¹ vs. 26.2 ± 8.4 ml min⁻¹; $P=0.42$) (Δ sweat rate tended ($P=0.09$) to be greater in the Cr group). Total sweat loss was not significantly different between trials in either the Cr (1.5 ± 0.4 L vs. 1.4 ± 0.2 L; $P=0.17$) or placebo (1.4 ± 0.5 L vs. $1.3 \pm$

Figure 5.5

**Changes in plasma volume during pre and post supplementation in the Cr (top panel left) and placebo (top panel right) group. Changes in plasma volume (re-calculated for all groups using their respective [Hb] and [Hct] measured during the pre-supplementation tests as baseline) during pre and post supplementation in the Cr (bottom panel left) and placebo (bottom panel right) group.
* Indicates a significant difference post-supplementation compared to baseline**



● Pre-supplementation
○ Post-supplementation

0.5 L; P=0.81) groups. Both sweat rate and sweat loss were similar pre-compared to post-supplementation in the "responders".

Blood Metabolite Concentrations at rest and during Exercise

Resting blood metabolite concentrations were not different between groups or following supplementation (Table 5.3). During exercise (all time points), blood [glucose] and [lactate] increased compared to rest and there were no differences between groups or following supplementation (Table 5.3). Blood [glucose] and [lactate] also increased in both trials in the "responders" with no difference between the trials.

Plasma Volumes Changes

Plasma volume fell by 11 - 14% within the first 10 min of exercise and thereafter remained largely unchanged; there were no differences between conditions or following supplementation (Figure 5.5). However, there was a near-statistically significant increase in [Hb] post Cr-supplementation compared to pre-supplementation (P=0.055) (Δ [Hb] was not different). No such effect was observed in the "responders" or placebo groups. Plasma volume changes during exercise following supplementation were also calculated for all groups using their respective [Hb] and [Hct] measured during the pre-supplementation tests as baseline, assuming no change in red cell mass during the 7 day supplementation regimen (Fortney *et al*, 1981). Using this method of analysis,

plasma volume decreased to a greater extent at almost all measured time points post-supplementation compared to pre-supplementation in the Cr group, with no such finding in the placebo group (Figure 5.5).

Correlation analysis

In the Cr group, estimated Cr uptake was positively correlated with Δ body mass ($r=0.68$, $n=11$; $P=0.021$) and Δ TTE ($r=0.75$, $n=11$; $P=0.008$). A significant positive correlation was found between Δ body mass and Δ TTE ($r=0.73$, $n=11$; $P=0.011$).

Side effects

In general, subjects tolerated the supplementation protocol well, with no reports of gastrointestinal distress or muscle cramping. Two subjects from each group correctly identified the treatment they were receiving, while all other subjects were unsure of the treatment they received.

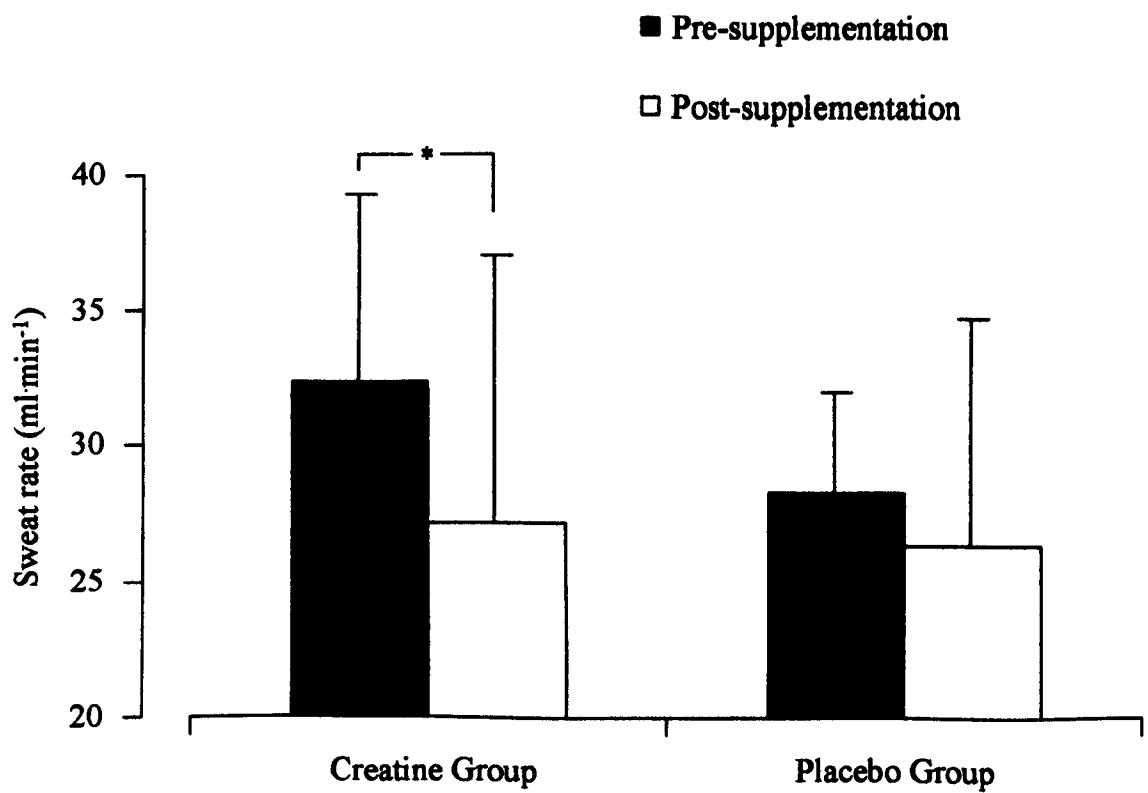
Table 5.3 Blood [Glucose] and [Lactate] for both groups pre and post-supplementation

Group	Trial	Exercise Time (min)										
		Rest	Unloaded	10	15	20	25	30	35	40	End	
[Glucose] (mmol/L)	Cr	Pre	4.8 ± 0.6	4.9 ± 0.5	4.8 ± 0.3	4.4 ± 0.5	4.5 ± 0.5	4.6 ± 0.4	4.5 ± 0.6	4.7 ± 0.6	4.9 ± 0.7	4.9 ± 0.7
	Post	5.0 ± 0.6	5.0 ± 0.6	4.9 ± 0.5	4.4 ± 0.7	4.5 ± 0.9	4.6 ± 0.9	5.0 ± 1.0	5.3 ± 1.0	5.3 ± 1.0	5.4 ± 1.2	
Plac	Pre	4.9 ± 0.5	4.8 ± 0.3	4.5 ± 0.2	4.1 ± 0.3	4.3 ± 0.4	4.4 ± 0.6	4.6 ± 0.7	4.7 ± 0.7	4.6 ± 0.6	5.4 ± 0.8	
	Post	5.0 ± 0.6	5.0 ± 0.2	4.6 ± 0.4	4.3 ± 0.7	4.5 ± 0.7	4.6 ± 0.8	4.8 ± 0.9	5.0 ± 1.1	5.1 ± 1.1	5.5 ± 1.3	
[Lactate] (mmol/L)	Cr	Pre	0.7 ± 0.2	0.8 ± 0.4	3.1 ± 1.6	3.0 ± 1.6	3.0 ± 1.6	3.1 ± 1.6	3.4 ± 1.6	3.5 ± 1.6	3.9 ± 1.6	4.4 ± 1.7
	Post	0.7 ± 0.4	0.9 ± 0.2	3.1 ± 1.7	2.9 ± 1.8	3.0 ± 1.8	3.3 ± 1.8	3.7 ± 1.6	3.9 ± 1.6	4.1 ± 1.5	4.8 ± 1.3	
Plac	Pre	0.7 ± 0.2	0.8 ± 0.3	4.1 ± 2.0	4.1 ± 2.2	4.3 ± 2.3	4.6 ± 2.3	4.8 ± 2.7	4.8 ± 2.4	4.8 ± 2.5	5.7 ± 2.9	
	Post	0.9 ± 0.4	0.9 ± 0.3	4.2 ± 2.5	4.1 ± 2.2	4.5 ± 2.4	4.5 ± 2.5	4.5 ± 2.6	4.5 ± 2.3	4.5 ± 1.9	5.9 ± 2.9	

Values are presented as the mean ± s.d.

Figure 5.6

Sweat rate responses pre and post
supplementation in the Cr and placebo group.
* Indicates a significant difference post-
supplementation compared to baseline



DISCUSSION

This study has demonstrated that a standard Cr supplementation regimen (Harris *et al*, 1992) increased TBW and ICW, reduced cardiovascular and some thermoregulatory (T_{rec}) responses during exercise, and increased exercise performance in a group of "responders" to Cr supplementation (pre to post). Cr supplementation resulted in a significant increase in TTE, but only in subjects whose intramuscular Cr uptake was significantly increased following the supplementation. A highly significant positive correlation was found between estimated Cr uptake and Δ TTE ($r=0.75$, $n=11$; $P=0.008$), indicating that subjects with the largest Cr uptake had the greatest performance gains. This finding is in agreement with other previously published studies (e.g. Casey *et al*, 1996; Experiment 1).

It is well established that there are "responders" and "non-responders" to Cr supplementation, with a proposed ergogenic threshold for intramuscular Cr uptake of 20 mmol kg⁻¹ dry muscle weight following Cr supplementation (Casey *et al*, 1996; Greenhaff *et al*, 1994). In Experiment 1, subdividing the Cr group into "responders" and "non-responders" on the basis of a physiological measurement (e.g., estimated muscle Cr uptake) confirmed the ergogenic potential of Cr supplementation. Failure to discriminate between those who respond and those who do not would therefore diminish any effect due to Cr supplementation. This may account for the confounding reports in the literature on the ergogenic potential of Cr supplementation. In the present study, 8

subjects were classed as "responders" based on estimated muscle Cr uptake. The average muscle Cr uptake for the "responders" was 53 ± 5 mmol kg⁻¹ dry muscle weight compared to Cr uptake ranging from 21-25 mmol kg⁻¹ dry muscle weight in the "non-responders". While these findings provide evidence consistent with an ergogenic threshold, assigning a specific threshold value is not possible as Cr uptake was only estimated and not directly measured in the present study. Nevertheless, estimated Cr uptake in the present study was very similar to other studies (Greenhaff *et al.*, 1994).

Cr supplementation in the present study was successful in attenuating cardiovascular and some thermoregulatory responses during exercise (i.e. decreased HR, T_{rec} , T_b , sweat rate). Metabolic rate during exercise was also reduced post-supplementation in the Cr group despite no differences in $\dot{V}O_2$, mechanical efficiency, and RER. This difference in metabolic rate may reflect the non-significant reduction in RER during exercise after Cr supplementation, possibly as a result of the significantly lower $\dot{V}CO_2$ post-Cr supplementation. Differences in substrate utilisation are unlikely to be the cause as there was no difference in blood metabolites. Although some of the differences in cardiovascular and thermoregulatory responses between the Cr and placebo groups were not detected in the "responders" (i.e., HR and sweat rate), possibly due to the smaller subject number, the increase in exercise performance found in the "responders" is most likely to be due to the Cr-induced attenuated physiological responses. Subjects who had supplemented with Cr reported, on

average, significantly lower ratings of perceived leg fatigue after 25 min of exercise, suggesting they were able to discern the benefit of this putative hyperhydration strategy. Five out of the eight "responders" reported that they found the post-supplementation trial to be easier; these same five subjects also showed the greatest estimated Cr uptake and performance gains.

From the measurements obtained and/or derived in the present study, one can only speculate on the potential mechanisms for the improvement in exercise performance in the heat following Cr supplementation. Many hyperhydration methods (e.g. plasma volume expansion, glycerol hyperhydration, fluid consumption) have been tested during conditions of heat stress in an attempt to enhance cardiovascular/thermoregulatory responses, and consequently, improve exercise performance. Despite considerable potential and some positive results (e.g., Galloway & Maughan, 2000; Anderson *et al*, 2001), these strategies have frequently failed to improve exercise performance (Latzka *et al*, 1997; Latzka *et al*, 1998; Watt *et al*, 2000). For example, Watt *et al*, (2000) demonstrated that a 13% acute plasma volume expansion, a level similar to that observed after heat acclimatisation, had no effect on core temperature, skin blood flow, heart rate or exercise performance. These authors concluded that plasma volume expansion might not be critical for changes in thermoregulation and exercise performance in the heat.

We have demonstrated that Cr supplementation is an effective hyperhydration strategy. Cr supplementation has previously been shown to increase body mass

(Balsom *et al*, 1993; Greenhaff *et al*, 1994; Green *et al*, 1996; Experiment 1; Experiment 2), although whether this is due to an increase in water content associated with an increase in protein synthesis (Ingwall *et al*, 1974), or a result of water retention causing an increase in protein synthesis through cell swelling (Haussinger *et al*, 1993) is unclear. Recently, Saab *et al*, (2002), using magnetic resonance imaging, attempted to resolve this by examining the impact of Cr supplementation on water compartments within skeletal muscle using the transverse relaxation distribution of skeletal muscle to model water compartments within the cell and/or tissue. An increase in ICW was found to be the primary cause of the initial changes in body mass during Cr supplementation, but these authors were unable to identify the exact mechanism behind this increase. In the present study, supplementation with Cr was successful in increasing TBW by an estimated 800 ml on average, of which 600 ml could be accounted for by the increase in ICW. The increase in plasma volume was approximately 60 ml assuming 7.5% of this is plasma (Latzka & Sawka, 2000). This increase in plasma volume is unlikely to be of physiological significance. The overall level of dehydration attained by the end of exercise was not affected as indicated by a similar sweat loss and reduction in plasma volume between trials. This may be due to the attenuated physiological responses, or reflect the longer exercise time in the "responders" post-Cr supplementation.

Two other studies have assessed the effect of Cr supplementation on performance during exercise in the heat (Volek *et al*, 2001; Kern *et al*, 2001).

Volek *et al* (2001) examined the effects of Cr supplementation on acute cardiovascular, renal, temperature and fluid-regulatory hormonal responses during 35 min of exercise followed immediately by three 10 s maximal sprints on a cycle ergometer in the heat. Significant increases in body mass and peak power during all three 10 s sprints were found following Cr supplementation, compared to no change in the placebo group. No differences, however, were found in HR, blood pressure, and sweat rate following Cr supplementation. More recently, Kern *et al* (2001) examined the effects of 28 days of Cr supplementation on HR and T_{rec} during 60 min of cycling exercise at 60% $\dot{V}O_2$ max in the heat. Significantly greater gains in body mass and TBW were found compared to the placebo group, with a consequent attenuation of T_{rec} post-supplementation. The increases in body mass and TBW, and the attenuation in T_{rec} observed were of a similar magnitude as observed in the present study. However, subjects in the study by Kern *et al* (2001) exercised for a fixed period of time (i.e. 60 min) and the effects of Cr supplementation on exercise performance could, therefore, not be evaluated.

Previous studies investigating the effects of Cr supplementation on muscle bioenergetics during submaximal exercise have produced conflicting results, with two reports of no effect of Cr loading on whole-body $\dot{V}O_2$ during exercise of varying intensity (Balsom *et al*, 1993; Stroud *et al*, 1994), but also reduced $\dot{V}O_2$ and blood lactate accumulation during steady-state exercise at 50% Δ (Jones *et al*, 2002). Jones *et al* (2002) attributed the lower $\dot{V}O_2$ and blood lactate

accumulation following Cr supplementation to a change in the pattern of fibre-type recruitment and/or a reduction in the number of muscle fibres recruited. Within the present investigation, however, the absence of any differences in metabolic variables such as blood [lactate], RER and, importantly, $\dot{V}O_2$, would suggest that altered muscle bioenergetics is unlikely to be the primary determinant of the enhanced exercise performance in the heat following Cr loading.

A significant finding of the present study was the lower T_{rec} at exhaustion post-supplementation in the Cr group, and in the "responders" despite the longer exercise time to exhaustion. In contrast, subjects in the placebo group fatigued at a similar T_{rec} on the two trials which is in agreement with the results of many previous studies which have proposed the attainment of a critical high temperature, typically 39.6 °C, as the main factor limiting exercise performance in the heat (e.g. Nielsen *et al*, 1990; Nielsen *et al*, 1993). Other studies have shown fatigue to occur over a range of core temperatures (i.e. 38 - 40°C), implying that fatigue in the heat is multifactorial in aetiology (Latzka *et al*, 1998). A clear attenuation in the rise in T_{rec} was observed in the present study. Although the exact cause of this cannot be explained, specific heat of the individual may provide some insight. For example, 0.83 Kcal of heat production per kg of body mass is required to increase T_{rec} by 1°C, therefore an expansion of TBW resulting in an increase in body mass could lead to an increased distribution of heat within the body. Thus the overall temperature increase would be attenuated by water expansion.

The finding of a lower T_{rec} at exhaustion post-Cr supplementation in the present study, combined with the failure to characterise the fatigue process in terms of peripheral factors (e.g. similar blood metabolite levels), may suggest that a major central fatigue component may be involved.

Recently, the role of central neural mechanisms in the fatigue process during exercise in the heat has been studied (Nielsen *et al*, 2001; Pitsiladis *et al*, 2002). Nielsen *et al* (2001) suggested that fatigue may occur at a critical brain temperature. Nielsen *et al* (2001) observed changes in EEG activity in the frontal area of the brain, possibly indicating hyperthermia-associated fatigue. Whether hyperthermia induces fatigue directly via an increase in brain temperature or indirectly, via afferent signals originating from skeletal muscle, cardiac muscle or internal organs in response to a rise in local temperature is unclear. Further support for a central fatigue component is the observation that peripheral markers of central 5-HT activity, such as prolactin and cortisol, are elevated during exercise in the heat (Frewin *et al*, 1976; Pitsiladis *et al*, 2002). Pitsiladis *et al* (2002), showed that serum [prolactin] was significantly higher at exhaustion during exercise in the heat compared to exercise in the cold, and correlated with T_{rec} only during exercise in the heat. These findings provide evidence, although indirect, that the serotonergic system and/or another closely related neurotransmitter systems (e.g. the dopaminergic system) may be involved in fatigue during exercise in the heat. At present, it is unknown what effect Cr supplementation has on the CNS, and in particular, on thermoregulatory control. Cr supplementation has been shown to gradually increase brain [Cr],

indicating that the blood-brain barrier is partly permeable to Cr (Schulze *et al*, 1997). It remains to be determined what effect, if any, increased CNS [Cr] has on the central fatigue process.

CONCLUSIONS

In the present study, $22.8 \text{ g}\cdot\text{d}^{-1} \text{ Cr}\cdot\text{H}_2\text{O}$ for 7 days was effective in increasing predominantly ICW and reducing cardiovascular and thermoregulatory responses during prolonged exercise in the heat. The attenuation of these responses resulted in a significant increase in time to exhaustion, but this effect was only seen in subjects whose intramuscular Cr levels were significantly increased following Cr supplementation (i.e. "responders" to Cr supplementation).

CHAPTER SIX

(Experiment 4)

Effects of Short and Long Term Creatine Supplementation on Body Composition and Muscle Strength in Patients with Moderate to Severe COPD

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a disabling condition, which is predicted to become the 4th most common cause of death world-wide by 2020 (Lopez & Murrey, 1998). Skeletal muscle dysfunction, which adversely affects skeletal muscle strength (Hamilton *et al*, 1995; Gosselink *et al*, 1996) and mass (Arora & Rochester, 1982), is frequently observed in patients with COPD. These changes are recognised as important factors in COPD, as they directly contribute to the handicap and disability seen within this group. Other alterations in skeletal muscle metabolism have also been observed within this patient population, such as significantly longer half-time recovery of phosphocreatine following intense exercise as compared to a control group (Payen *et al*, 1993; Sala *et al*, 1999), and also studies showing lower resting levels of PCr (Gertz *et al*, 1977; Jakobsson *et al*, 1990), with these abnormalities possibly contributing to the overall exercise intolerance observed in patients with COPD.

Cachexia, another consequence of this disease, has also been shown to be an independent predictor of mortality in this patient population (Schols *et al*, 1998). In recent years, researchers have attempted to reverse abnormalities in muscle mass, muscle strength and muscle metabolism using numerous strategies ranging from appetite stimulators (Weisberg *et al*, 2002), anabolic steroids (Ferreira *et al*, 1998), to more recently recombinant human growth hormone

(rhGH) (Burdet *et al*, 1997). However, despite the majority of these strategies leading to an increase in muscle mass, there have been no observed increases in muscle strength (Burdet *et al*, 1997; Ferreira *et al*, 1998; Weisberg *et al*, 2002). For example, Burdet *et al* (1997) examined the effects of 0.15 IU·kg⁻¹ rhGH per day for 3 weeks on lean body mass, muscle strength and exercise tolerance in underweight patients with COPD. In this study, despite a significant increase in lean body mass (2.3 ± 1.6 kg), daily administration of rhGH did not increase maximal respiratory pressures, handgrip strength, maximal exercise capacity or subjective well being.

Creatine (Cr) supplementation has been shown in the majority of studies carried out in healthy subjects to have the ability to alter skeletal muscle metabolism (Greenhaff *et al*, 1994; Balsom *et al*, 1995), and to increase body mass (Greenhaff *et al*, 1994; Balsom *et al*, 1995; Experiment 1-3), fat-free mass (Kreider *et al*, 1998; Becque *et al*, 2002; Experiment 1), muscle strength (Kreider *et al*, 1998; Maganaris & Maughan, 1998; Experiment 1; Experiment 2) and muscle endurance (Experiment 1). Researchers have also examined the effects of Cr supplementation on body composition and muscle strength in older subjects (i.e. with age-related alterations in their skeletal muscle metabolism), with the majority of studies indicating an ergogenic effect of Cr loading on body composition (Jakobi *et al*, 2001; Gotshalk *et al*, 2002), muscle endurance (Rawson *et al*, 1999; Gotshalk *et al*, 2002), muscle strength (Gotshalk *et al*, 2002) and also

when used in conjunction with a resistance training programme (Chrusch *et al*, 2001). For example, Gotshalk *et al* (2002) examined the effects of 7 days of Cr supplementation ($0.3 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) on body mass, FFM (estimated using hydrostatic weighting), maximal dynamical strength (upper and lower body), maximal isometric strength and lower extremity functional capacity in normally active older men (59 - 72 yr). The results of this study (Gotshalk *et al*, 2002) indicated that 7 d of Cr supplementation was effective at increasing several of the indices of muscle performance, including functional tests, with no adverse side effects. However, two studies have indicated that Cr supplementation does not have a beneficial effect on performance in older subjects (Bermon *et al*, 1998; Jakobi *et al*, 2001). For example, Bermon and co-workers (1998) indicated that Cr supplementation had no effect on body composition, maximal dynamical strength, dynamical and isometric endurance in healthy elderly subjects, whether or not it was associated with an effective strength training programme.

Based on this work in relation to both healthy young and older populations and also on the proposed benefits of Cr supplementation on body composition, muscle function and muscle metabolism, some researchers have examined the role of Cr supplementation in patient populations. Previously, researchers have examined the effects of this putative ergogenic aid in patients with chronic heart failure (CHF) (Gordan *et al*, 1995; Andrews *et al*, 1998), myasthenia gravis (Stout *et al*, 2001), rheumatoid arthritis (Willer *et al*, 2002) and patients with

mitochondrial cytopathies (Tarnopolsky *et al*, 1997), for all of which positive benefits of Cr supplementation on body composition and/or skeletal muscle function have been reported.

The aim of the present study was therefore two-fold: firstly, to determine the effects of Cr loading on upper and lower body strength, upper and lower body strength endurance and body composition; and, secondly, to examine the effects of Cr supplementation in conjunction with standard pulmonary rehabilitation on the above-mentioned variables in a group of patients with moderate to severe COPD.

METHODS

Patients

Twenty-nine patients (11 females and 18 males) with established clinical and functional diagnosis of moderate-to-severe COPD ($FEV_1 < 60\%$ predicted and FEV_1/FVC ratio $< 70\%$) (American Thoracic Society, 1987) comprised the study group (Table 6.1). Inclusion criteria were absence of locomotor or neurological diseases, and no change in medication dosage or exacerbation of symptoms in the preceding four weeks. All patients were optimised in terms of standard medical therapy: maintenance medications included short and long acting β_2 -agonists, anticholinergics, theophylline, and inhaled steroids. Patients were excluded if they had taken oral prednisolone within the preceding four weeks. The patients' eligibility was assessed by interview prior to their informed consent for participation in the study. Before the tests, the procedures, including the known risks, were described in detail and written informed consent (as approved by the North Glasgow Hospital Ethics Committee) was obtained from all patients.

Table 6.1: Baseline Characteristics of the two groups of patients

	Placebo Group (n=15)	Creatine Group (n=14)
	Baseline	Baseline
Age (yr)	65 ± 10	61 ± 8
Height (m)	1.64 ± 10	1.62 ± 6
FVC (L)	2.7 ± 0.8	3.0 ± 0.6
FEV ₁ (L)	1.1 ± 0.4	1.1 ± 0.3
FEV ₁ (% pred)	42.5 ± 14.6	44.4 ± 14.4
FEV ₁ /FVC (%)	38.9 ± 8.4	36.4 ± 11.2
TLC (% pred)	128.0 ± 17.4	124.6 ± 18.7

Values are presented as the mean ± s.d

Definition of abbreviations: FVC: forced vital capacity; FEV₁: forced expiratory volume in one second; TLC: total lung capacity; pred: predicted values.

Experimental Design

Prior to the commencement of the experimental trials, patients visited the laboratory in order to become familiar with the isokinetic and handgrip dynamometer. Familiarisation trials were carried out until the variability of three consecutive performances was within 5% for peak isokinetic force at a speed of $70^{\circ}\cdot\text{s}^{-1}$. The test-retest reliability of isokinetic assessments performed in our laboratory has revealed a high intra-class correlation (ICC) for strength measures (Isokinetic force ICC = 0.92 ($70^{\circ}\cdot\text{s}^{-1}$)), these test-retest reliability values are based on subjects having each undergone two familiarisation tests). Patients were assigned in a double blind fashion to either a Cr group or a placebo group, stratified according to their body composition: body mass index (BMI) and/or fat-free mass index (FFMI) with random assignment so that one member of each pair was in the Cr group and the other in the placebo group. This was done by dividing the patients into depleted and non-depleted patients, with patients considered as nutritionally depleted if they had a BMI of < 21 or an FFMI of < 15 (females) or < 16 (males) (Van Itallie *et al*, 1990). Following the familiarisation period, all patients performed three tests (baseline, post-loading and post-rehabilitation). The first test was conducted at least 48 hours after the familiarisation trial, and the final test was conducted at least 48 hours after the final rehabilitation session.

The Cr group ingested $17.1 \text{ g}\cdot\text{d}^{-1} \text{ Cr}\cdot\text{H}_2\text{O}$ (equivalent to $5 \text{ g Cr} \times 3$ times daily) for the first 14 days (loading). From day 15, patients consumed $5.7 \text{ g}\cdot\text{d}^{-1} \text{ Cr}\cdot\text{H}_2\text{O}$ (equivalent to 5 g Cr daily) for the remainder of the study (maintenance). This maintenance dose was selected on the basis of published work by Hultman *et al* (1992) showing $2 \text{ g}\cdot\text{d}^{-1} \text{ Cr}\cdot\text{H}_2\text{O}$ was adequate in maintaining elevated muscle PCr stores in patients not involved in strenuous exercise. As the patients in the present study were training twice a week at high intensities, it was decided to increase the maintenance dose to $5 \text{ g}\cdot\text{d}^{-1} \text{ Cr}\cdot\text{H}_2\text{O}$ in an attempt to maintain muscle PCr stores. The addition of glucose to the Cr has been shown to significantly enhance the uptake of Cr (Green *et al*, 1996; Steenge *et al*, 1998). Patients were instructed to ingest the supplements at equal intervals throughout the day. The placebo group consumed $120 \text{ g}\cdot\text{d}^{-1}$ of glucose polymer ($40 \text{ g} \times 3$ times daily) for the first 14 days, followed by 40 g a day for the subsequent duration of the study. The placebo group followed the same procedure as the Cr group with regard to the preparation of the supplements. Both supplements had similar taste, texture and appearance and were placed in generic containers to ensure double-blind administration. Patients were also requested to eliminate caffeine and caffeine containing foods from their diet over the loading phase to minimize the possible inhibitory effects of caffeine on the ergogenic effect of Cr (Vandenberghe *et al*, 1996). At the end of the study, all patients gave verbal assurance that they had complied with all instructions.

Procedures

Patients reported to the laboratory on the day of testing, after having refrained from strenuous activity the day before. Following the measurement of stature and body mass, body composition was measured using bioelectrical impedance.

Following the measurements of body composition, patients underwent a standardised warm-up on the isokinetic dynamometer. Patient's isokinetic peak torque ($70^{\circ}\cdot\text{s}^{-1}$) was then measured during 5 repetitions using a Kin-Com II isokinetic dynamometer (Chattecx Corporation, Chattanooga, USA). The dominant leg was tested. The position of the patients on the isokinetic dynamometer was standardised; the anatomical axis of the knee joint was aligned with the rotational axis of the dynamometer by adjusting the seat position and the lever head of the dynamometer. Individual seat length and height were recorded for each patient and used in subsequent tests. Patients were held in the seat position with Velcro belts around the waist, thigh and lower leg proximal to the ankle, this also allowed for complete isolation of the testing leg. During the measurement, patients had their arms crossed over their chest while their non-involved leg and upper body was kept stationary. Patients were instructed to exert maximal effort throughout the full range of motion during each repetition. Verbal encouragement was given to maximise

performance. The same investigator conducted all tests. Having completed the peak torque isokinetic testing and following a 5 min recovery period, patients went on to complete five sets of 15 repetitions at a speed of $150^{\circ}\cdot\text{s}^{-1}$ as a measure of strength endurance of the quadriceps. Patients were given 2 min rest between sets (Greenhaff *et al.*, 1994). Following a further 10 min rest, handgrip strength and strength endurance was tested on there dominant and non-dominant hand. Following a handgrip specific warm-up, patients completed 5 maximal voluntary isometric contractions with 30 s rest between each contraction, patients dominant hand was always tested first followed by there non-dominant hand, with the order kept the same for all subsequent tests. Having completed this measure of grip strength, patients went on to perform 3 sets of contractions to exhaustion with the intensity set at 70% of their pre-determined 1 repetition maximum (1RM). Again the dominant hand was tested first, with patients receiving 2 min rest between each set of contractions (Greenhaff *et al.* 1994). Fatigue was defined as the failure to exert the required intensity in 3 contractions in a row. Patients received verbal encouragement throughout all testing. Following two weeks of loading, patients returned to the laboratory and completed the same tests as on there baseline measurement day. Patients then entered a standard pulmonary rehabilitation programme for 8 wk, and following this 8 wk of training returned to the laboratory to complete the battery of tests identical to those at baseline and post-loading.

Body Composition Estimation

Body composition was performed on the same day as the strength measures by the same investigator. Height was measured (to the nearest .01 m) using a stadiometer, with patients standing barefoot. Body mass was assessed (to the nearest 0.1 kg) with patients wearing only a swimsuit.

Bioelectrical Impedance (BIA)

Measurement of FFM by bioelectrical impedance (FFM_{BIA}) (Bodystat-5000, Bodystat Ltd, Isle of Man, UK) was performed on the right side, with patient's supine, and with their limbs slightly apart from the trunk. After the skin had been cleaned with 70% alcohol, two injector electrodes were placed on the dorsal surface of the right hand and foot, and two detector electrodes were placed between the radius and ulna and on the ankle between the medial and lateral malleoli. The impedance to current flow (50 kHz) between the injector and detector electrodes was determined. A patient-specific prediction equation based on resistance (R), body mass (BM), height (H) and sex (S, males =1 and females = 0) was used to determine FFM (Kyle *et al*, 1998):

$$\text{FFM (kg)} = -6.06 + (H \times 0.283) + (BM \times 0.207) - (R \times 0.024) + (S \times 4.036).$$

Pulmonary Rehabilitation Programme

The programme consisted of 2 weekly sessions of 1 hr for 8 wks (i.e. 16 sessions in total). The exercise was conducted by a physiotherapist and consisted of a warm-up, multimodality upper and lower limb exercises such as walking, stair climbing, cycle ergometry, upper and lower body strength exercises, and breathing exercises with appropriate rest intervals between activities. The intensity and load of all exercises were individualised, with training targeted so that patients exercised at, or just below, the Borg breathlessness rating corresponding to their symptom-limited maximal rating; based on these ratings, subjects trained at the highest attainable work level during each session (e.g. progression and overload principle). Patients were also given a copy of alternative exercises, so they could train at home.

Prior to the start of each exercise session, all patients underwent a standardised warm-up which comprised light intensity exercise for 5 - 10 min, followed by a series of stretches with an emphasis on stretching the musculature associated with the exercises that were to follow. The training intensity was progressively increased as patients successfully completed the required number of sets and repetitions. Patients kept training logs throughout the duration of the study detailing rating of dyspnoea during exercise sessions and the weight, sets and

repetitions lifted during strength training of the upper and lower extremities. Exercise sessions lasted on average 60 min (including the 10 min warm-up and 10 min cool-down). The compliance of the subjects was good, with only 4 withdrawals during the training period due to non-compliance, however five other patients dropped out during the pulmonary rehabilitation period due to an increased number of exacerbation's. The pulmonary rehabilitation programme carried out at Glasgow Royal Infirmary is in line with the general guidelines set out by British Thoracic Society position statement on Pulmonary rehabilitation in 2001 (BTS, 2001).

Data Analysis

Data were expressed as the mean \pm s.d. following a test for the normality of distribution. Statistical analysis was carried out using two factor ANOVA for repeated measures, followed by Student's *t*-test for paired data and two sample *t*-test for unpaired data, as appropriate. Pearson correlation analysis was used to assess the relationship between variables. Statistical significance was declared when $P < 0.05$.

RESULTS

1. Cr Loading (14 days)

Physical Characteristics

The physical characteristics of the two groups of patients were not significantly different before supplementation (Table 6.2). In the Cr group, body mass increased significantly from 60.9 ± 11.5 kg to 61.9 ± 11.2 kg following Cr loading ($P < 0.001$), with no change in the placebo group (66.7 ± 22.2 kg to 66.8 ± 22.0 kg, $P = 0.73$). The magnitude of change in body mass was significantly greater in the Cr group compared to the placebo group ($P = 0.002$) (Table 6.2). In the Cr group, FFM increased significantly post-loading (Table 6.2), with no increase observed in the placebo group (Table 6.2). The change in FFM over the supplementation period was significantly greater in the Cr group compared to the placebo group (Table 6.2).

There was no significant increase in body fat as estimated by BIA in the placebo group following loading (Table 6.2), in the Cr group there was an observed increase in body fat as estimated by BIA ($P = 0.015$). However, the magnitude of this increase was not significantly greater when compared to the placebo group ($P = 0.18$).

Table 6.2: Body composition changes of the two groups of patients following Cr loading

	Placebo Group (n=15)		Creatine Group (n=14)	
	Pre	Post-loading	Pre	Post-loading
Body mass (kg)	66.7 ± 22.2	66.8 ± 22.0	60.9 ± 11.5	61.9 ± 11.2*†
FFM (kg)	43.7 ± 10.1	43.8 ± 10.2	41.1 ± 7.4	41.8 ± 7.0*†
FM (kg)	23.0 ± 14.0	23.0 ± 13.7	19.8 ± 7.1	20.1 ± 7.2*

Values are presented as the mean ± s.d.

* Indicates a significant difference from pre-supplementation values; † indicates a significant difference between the change in the Cr group compared to the placebo group

Lower Body Strength and Endurance

Average peak torque (average of the 5 repetitions) increased significantly following Cr loading in the Cr group (86.7 ± 27.8 Nm to 90.1 ± 26.6 Nm, $P=0.001$), with no increase in the placebo group (79.0 ± 32.3 Nm to 78.0 ± 32.1 Nm, $P=0.376$). The magnitude of the increase was significantly greater than that observed in the placebo group ($P=0.0032$) (Table 6.3).

Peak torque (highest score obtained during the 5 repetitions) also significantly increased following supplementation in the Cr group (90.6 ± 27.8 Nm to 93.6 ± 26.6 Nm, $P<0.001$), with no increase observed in the placebo group (83.5 ± 32.9 Nm to 82.0 ± 33.8 Nm, $P=0.246$). The magnitude of the increase observed regard to peak torque was significantly greater in the Cr group compared to the placebo group ($P=0.0036$) (Figure 6.1a).

The Cr group produced significantly greater total work (TW) during all 5 sets of 15 contractions ($P=0.032$, $P=0.006$, $P=0.012$, $P=0.022$ and $P=0.016$, respectively) following loading. In the placebo group patients produced similar amounts of work pre and post-loading ($P=0.202$, $P=0.788$, $P=0.774$, $P=0.134$ and $P=0.091$, respectively). When the magnitude of change was compared between both groups, the Cr group improved to a greater extent than the placebo group in 4

out of the 5 sets (set 2: $P=0.0059$, set3: $P=0.012$, set 4: $P=0.014$ and set 5: $P=0.0099$) (Figure 6.2a).

Combined TW (all 5 sets added together) increased from 1996 ± 746 J to 2471 ± 764 J ($P=0.014$) in the Cr group, compared to no significant increase in the placebo group (1670 ± 855 J to 1675 ± 835 J, $P=0.516$). The magnitude of the increase was significantly greater in the Cr group, compared to the placebo group (475 ± 626 J vs. -13.1 ± 76.4 J, $P=0.012$) (Figure 6.3a).

Upper Body Strength and Endurance

There was no significant increase in average or peak hand grip strength in either the Cr or placebo group following supplementation (as measured on both right and left hand) (Table 6.3). There was no significant increase in the number of repetitions performed post-loading, compared to pre-loading, in the placebo group in any of the 3 sets on the right or left hand. In the Cr group, patients produced a significantly greater number of repetitions in sets 2 (14.9 ± 5.8 to 17.4 ± 7.1 , $P=0.006$) and 3 (11.8 ± 4.3 to 13.6 ± 4.7 , $P=0.009$) following supplementation on their right hand. Similar findings were observed with regard to patients left hand also (sets 2: 14.4 ± 5.0 to 16.5 ± 4.4 , $P=0.008$ and set 3: 11.5 ± 4.0 to 13.6 ± 4.0 , $P=0.005$). The magnitude of the increase was significantly greater in the Cr group, compared to the placebo group, in set 2 only ($P=0.044$), with a strong

tendency observed in set 3 ($P=0.051$) with regard to the right hand. While on the left hand the magnitude of the increase was significantly greater during set 2 ($P=0.008$) and set 3 ($P=0.007$) (Figure 6.4). Total repetitions (sum of all 3 sets) was significantly greater in the Cr post-loading compared to the placebo group in both the left and right hand (Table 6.3).

Table 6.3: Changes in strength characteristics of the two groups of patients following Cr loading

	Placebo Group (n=15)		Creatine Group (n=14)	
	Pre	Post-loading	Pre	Post-loading
Peak Handgrip Strength Left (kg)	24.9 ± 11.4	24.7 ± 10.8	24.9 ± 8.1	25.2 ± 8.0
Peak Handgrip Strength Right (kg)	26.2 ± 10.5	25.8 ± 10.4	27.7 ± 8.2	28.2 ± 8.7
Average Peak Torque (Nm)	79.0 ± 32.3	78.0 ± 32.1	86.7 ± 27.8	90.1 ± 26.6* †
Total Reps Handgrip Left	40.5 ± 15.6	39.8 ± 15.1	46.9 ± 13.3	51.2 ± 13.5* †
Total Reps Handgrip Right	50.7 ± 17.8	51.5 ± 17.1	48.8 ± 15.5	53.4 ± 18.4* †

Values are presented as the mean ± s.d.

* Indicates a significant difference from pre-supplementation values; † indicates a significant difference between the change in the Cr group compared to the placebo group

2. Cr Supplementation Combined with Pulmonary Rehabilitation

Following the two weeks of Cr, patients entered a standard 8-week pulmonary rehabilitation programme. However, due to an increase in the number of exacerbations and non-compliance to the pulmonary rehabilitation programme, only 21 patients completed this phase of the study (11 patients in the Cr group and 10 in the placebo group).

Physical Characteristics

In the Cr group, there was no significant change in body mass following supplementation and rehabilitation (Table 6.4) or when confined to the change from post-loading to the end of rehabilitation (rehab-load) (62.8 ± 12.6 kg to 61.8 ± 13.9 kg, $P=0.079$). In the placebo group, again, there was no significant change in body mass following supplementation and rehabilitation ($P=0.447$) or as measured from rehab-load ($P=0.486$).

In the Cr group, FFM increased significantly over the supplementation/rehabilitation period, with no change in the placebo group (Table 6.4). In the placebo group, no changes in FFM were observed over this period (Table 6.4).

Table 6.4: Body composition changes of the two groups of patients following rehabilitation

	Placebo Group (n=10)		Creatine Group (n=11)	
	Pre	Post-rehabilitation	Pre	Post-rehabilitation
Body mass (kg)	77.0 ± 20.0	76.7 ± 20.2	61.9 ± 12.9	61.8 ± 13.9
FFM(kg)	47.9 ± 9.1	48.4 ± 9.2	41.7 ± 8.1	43.1 ± 9.0*
FM (kg)	29.1 ± 12.7	28.4 ± 12.7*	20.2 ± 7.4	18.7 ± 7.8* †

Values are presented as the mean ± s.d.

* Indicates a significant difference from pre-supplementation values; † indicates a significant difference between the change in the Cr group compared to the placebo group

There was a significant reduction in FM over both the supplementation/rehabilitation and rehab-load periods in the Cr group, with this reduction only seen over the supplementation/rehabilitation period in the placebo group (Table 6.4). As a result, the magnitude of the reduction was significantly greater in the Cr group over both periods, when compared to the placebo group (Table 6.4).

Upper Body Strength Endurance

Average hand grip strength (right and left hand) increased following supplementation/rehabilitation in the Cr group and also when measured from rehab-load. In the placebo group, there was no significant increase over the supplementation/rehabilitation period ($P=0.227$) for either hand, but there was a significant increase over the rehab-load period ($P<0.001$) with regards to the right hand. The magnitude of the increases were significantly greater over both periods ($P=0.034$ and $P=0.024$, respectively) in the Cr group, compared to the placebo group, but only in the right hand (dominant hand).

Peak hand grip strength (right and left) also increased significantly over both the supplementation/rehabilitation and rehab-load periods in the Cr group. In contrast, for the placebo group, an increase was observed only over the rehab-load period ($P=0.002$) on the right hand. The magnitude of the increase was

Table 6.5: Changes in strength characteristics of the two groups of patients following Cr and rehabilitation

	Placebo Group (n=10)		Creatine Group (n=11)	
	Pre	Post-loading	Pre	Post-loading
Peak Handgrip Strength Left (kg)	27.5 ± 11.9	28.5 ± 11.1	25.2.0 ± 8.2	27.6 ± 8.5
Peak Handgrip Strength Right (kg)	28.8 ± 10.6	29.4 ± 10.4	28.0 ± 9.1	30.8 ± 9.5* †
Average Peak Torque (Nm)	88.7 ± 28.0	103.0 ± 27.6*	86.1 ± 30.0	107.8 ± 29.1* †
Total Reps Handgrip Left	46.8 ± 13.5	52.9 ± 14.5*	47.6 ± 14.9	68.9 ± 17.9* †
Total Reps Handgrip Right	52.2 ± 19.4	60.3 ± 20.0*	49.0 ± 17.1	66.2 ± 21.9* †

Values are presented as the mean ± s.d.

*Indicates a significant difference from pre-supplementation values; † indicates a significant difference between the change in the Cr group compared to the placebo group

significantly greater only over the supplementation/rehabilitation period ($P=0.040$) in the Cr group, compared to the placebo group, but only with regard to the right hand (Table 6.5).

Total repetitions (sum of all 3 sets) (right and left hands) was significantly greater in the Cr and placebo groups following supplementation/ rehabilitation and rehab-load, with the magnitude of the increase being significantly greater in the Cr group, compared to the placebo group over both periods (Table 6.5).

Lower Body Strength and Endurance

Peak torque increased significantly in both the Cr and placebo groups following supplementation/rehabilitation ($P<0.001$ and $P<0.001$) and from rehab-load ($P<0.001$ and $P<0.001$). The magnitude of the change was significantly greater during both periods in the Cr group, compared to the placebo group ($P=0.020$ and $P=0.050$) (Figure 6.1b).

Average peak torque also increased significantly in both the Cr and placebo groups following supplementation/rehabilitation ($P<0.001$ and $P<0.001$) and from rehab-load ($P<0.001$ and $P<0.001$), with the magnitude of the change being significantly greater during both periods in the Cr group compared to the placebo group ($P=0.009$ and $P=0.030$, respectively).

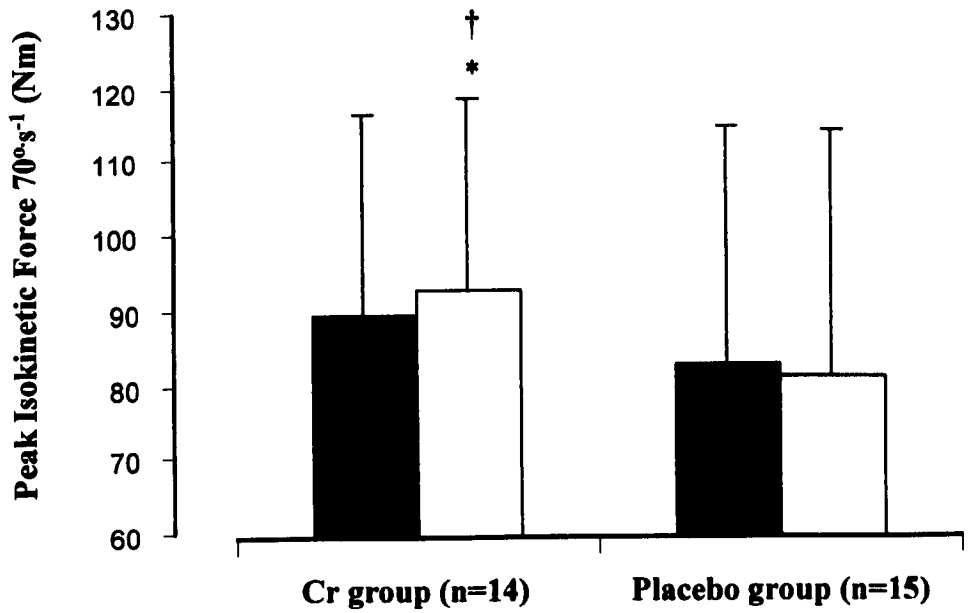
The Cr group produced significantly greater total work (TW) during all 5 sets of 15 contractions following supplementation/ rehabilitation. In the placebo group patients produced significantly greater TW during sets 2 and 3, with strong tendencies during the final two sets also (sets 3 and 5). When the magnitude of change was compared between both groups, the Cr group improved to a greater extent than the placebo group in all 5 sets (Figure 6.2b).

Combined TW (all 5 sets added together) significantly increased in the Cr following supplementation/rehabilitation ($P=0.002$) and during the rehab-load period ($P=0.001$), a strong tendency was observed in the placebo group to have increased TW over the supplementation/rehabilitation period ($P=0.054$) and there was a significant increase over the rehab-load period ($P=0.034$). The magnitude of the increase was significantly greater in the Cr group compared to the placebo group over both periods ($P=0.014$ and $P=0.042$, respectively) (Figure 6.3b).

Figure 6.1

Peak Isokinetic Force in the Cr and Placebo supplemented groups post-loading (top panel) and post-rehabilitation (bottom panel) * indicates a significant difference from pre to post supplementation. † indicates a significant greater change in the Cr group compared to the placebo group

A



■ Pre-supplementation
□ Post-supplementation

B

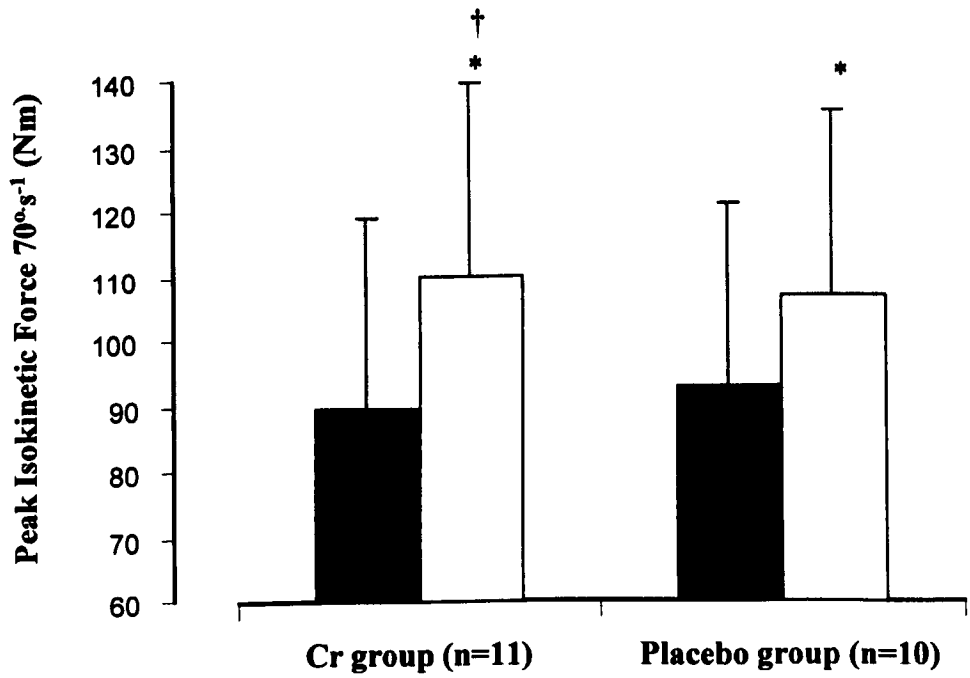
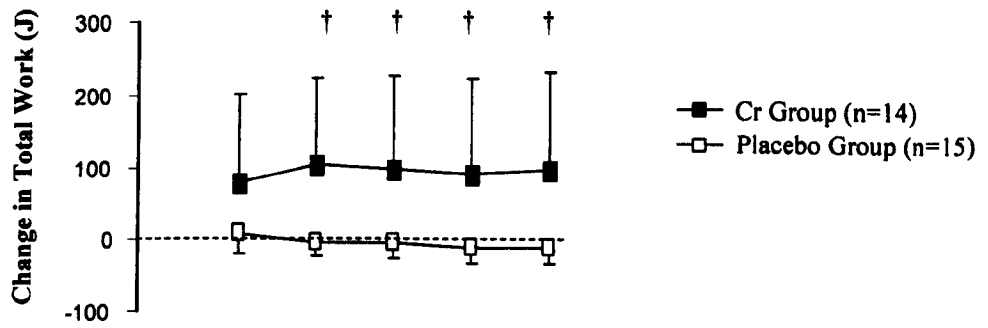


Figure 6.2

Change in Total in the Cr and Placebo supplemented groups post-loading (top panel) and post-rehabilitation (bottom panel). † indicates a significant greater change in the Cr group compared to the placebo group.

A



B

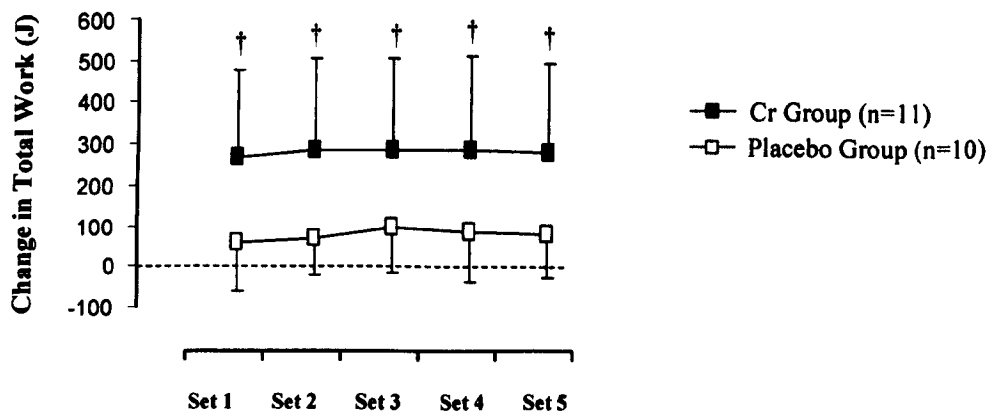


Figure 6.3

Combined Total Work (sets 1 to 5) in the Cr and Placebo supplemented groups post-loading (top panel) and post-rehabilitation (bottom panel). * indicates a significant difference from pre to post supplementation. † indicates a significant greater change in the Cr group compared to the placebo group

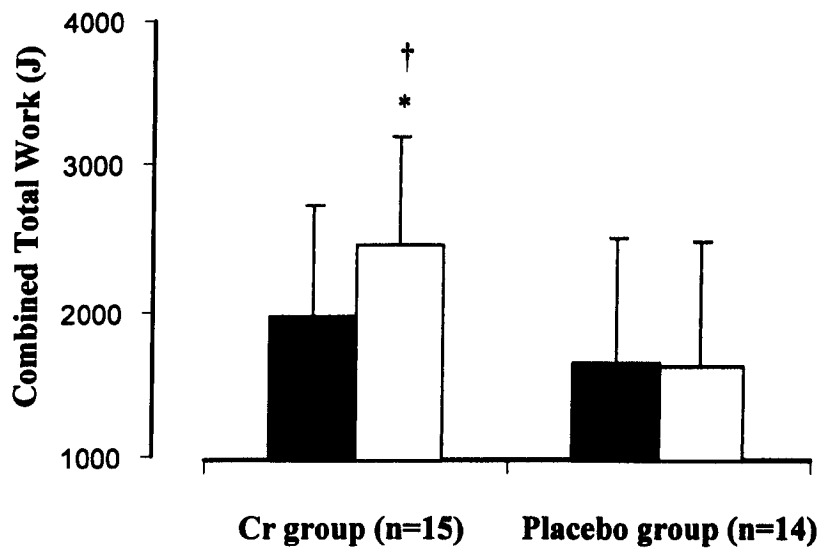
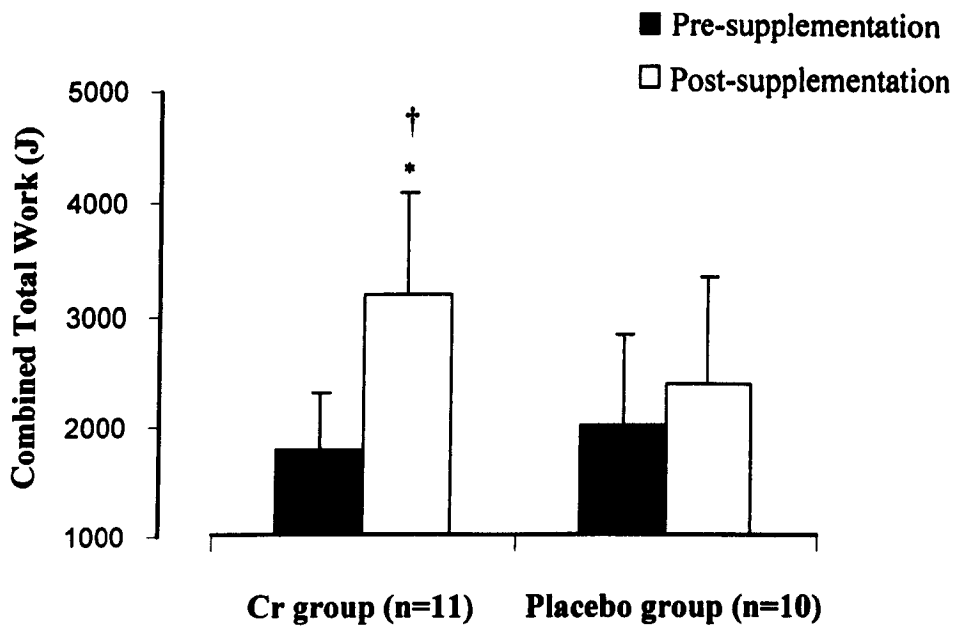
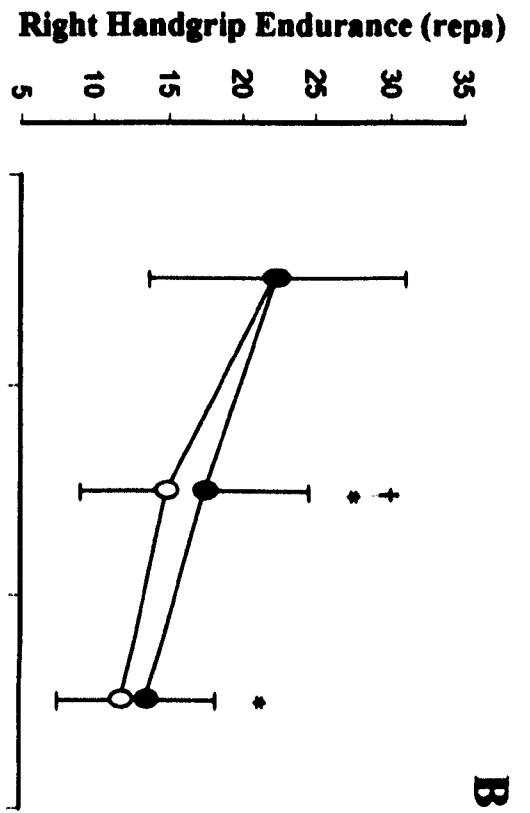
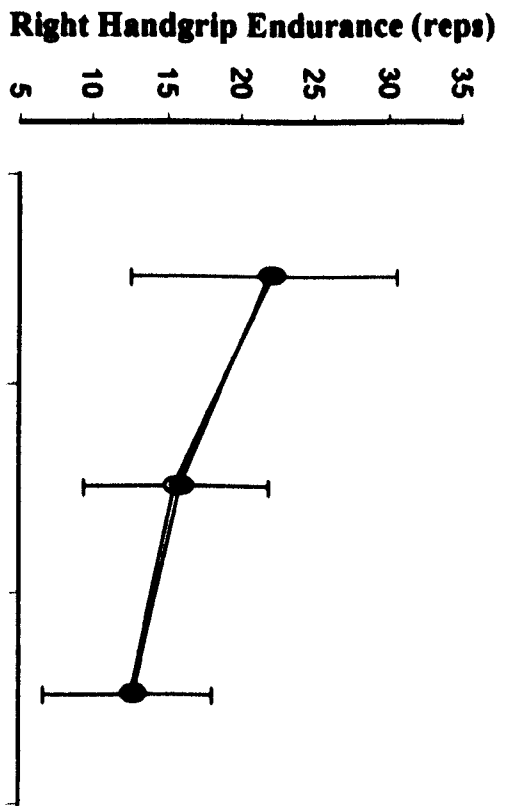
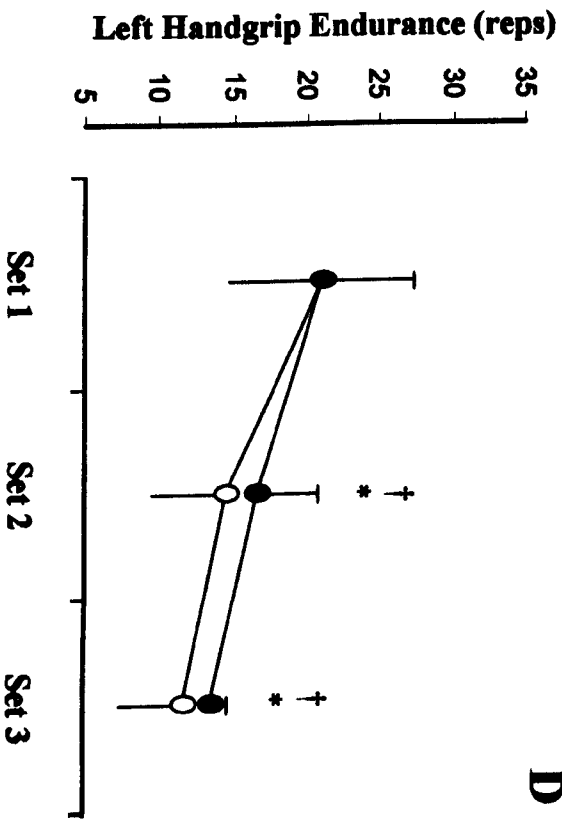
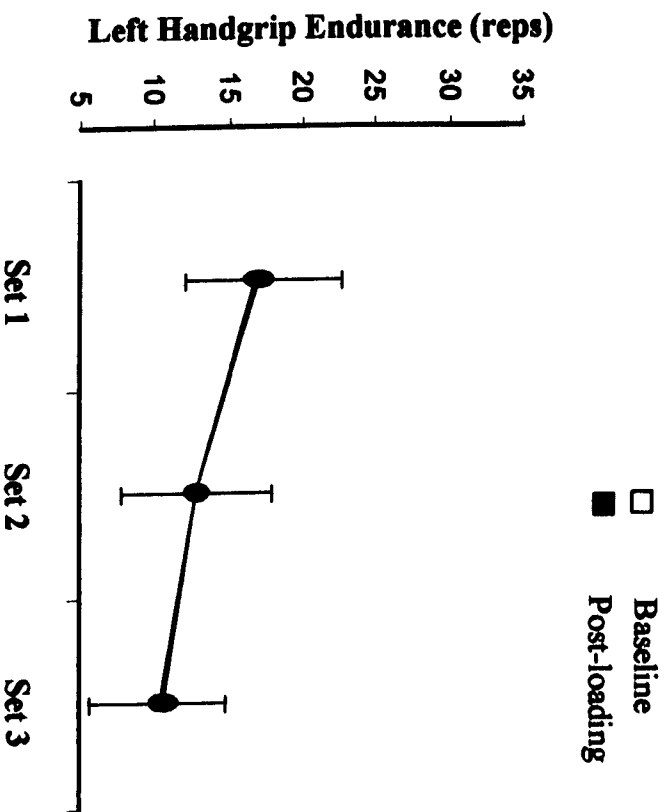
A**B**

Figure 6.4

Handgrip Endurance for right (top panels) and left hands (bottom panels) in the Cr (left side) and Placebo (right side) supplemented groups post-loading (top panel) and post-rehabilitation (bottom panel). * indicates a significant difference from pre to post supplementation. † indicates a significant greater change in the Cr group compared to the placebo group

A**B****C****D**

DISCUSSION

The results of the present study demonstrate that 14 days of oral Cr supplementation ($15 \text{ g}\cdot\text{d}^{-1}$) was effective in increasing body mass, FFM, lower body muscle strength, upper and lower body muscle endurance in patients with moderate to severe COPD during 14 days of supplementation. Additionally, this study also highlights a potential role for Cr supplementation when combined with a standard pulmonary rehabilitation programme. With respect to this aspect of the study, Cr supplementation combined with a pulmonary rehabilitation programme was more effective in increasing muscle strength (lower and upper body), muscle endurance (lower and upper body), FFM and FM (significant decrease) than pulmonary rehabilitation alone.

The present study demonstrated a significantly greater increase in body mass and, more importantly, FFM following short-term Cr supplementation (loading) compared to the placebo group. While there was no significant increase in body mass over the whole duration of the study (supplementation/rehabilitation), this was probably due to the observed decrease in FM, while the significant increase in FFM remained. The importance of this finding can be seen from a recent study by Schols *et al* (1998). In that study the authors demonstrated that patients whose body weight increased significantly following intervention ($> 2\text{kg}/8 \text{ wks}$) had a decreased mortality risk as compared to patients whose body weight did not

increase following this intervention. In this study, the researchers retrospectively analysed 400 patients with COPD, and they found weight gain (> 2kg/8 wk) in both depleted and non-depleted patients were significant predictors of survival, and also as part of the same study they also reported that low BMI was a significant independent predictor of increased mortality. For these reasons, many researchers have examined numerous intervention strategies in an attempt to increase body mass and, more importantly, FFM in patients with COPD. These strategies ranged from nutritional intervention and appetite stimulators to, more recently, rhGH and anabolic steroids. For example, Burnet *et al* (1997) and Yeh *et al* (2002) used rhGH and an anabolic agent (Oxandrolone), respectively, to increase FFM in this patient group. However while both of these interventions were successful in increasing FFM, not all patients could tolerate these interventions, with many reporting a range of side-effects. In the present study, we have shown short-term and long-term Cr supplementation to be an effective strategy in increasing FFM in this patient population with patients reporting no treatment associated side-effects. The findings of the present study are in agreement with previously published work in healthy young subjects (Experiment 1; Experiment 2) and healthy older subjects (Gotshalk *et al*, 2002; Chrusch *et al*, 2001; Jacobi *et al*, 2001).

Another significant finding in the present study was a significant greater increase in lower body strength following Cr loading (14 days), with no such increase

observed in upper body strength during this period. While this might seem contradictory at first, it has been shown in the past that lower limbs are affected to a greater extent than the upper limb muscles with regard to muscle strength (Hamilton *et al*, 1995; Gosselink *et al*, 1996), with this difference attributed to the greater reduction in activity of the lower limbs compared to the upper limbs in this patient population.

To date, this is the first intervention study in COPD to show a positive outcome with regard to muscle strength (lower body). Previously, researchers have used such strategies as anabolic steroids and rhGH in an attempt to increase muscle strength in this patient group but with no significant effect. For example, in the study by Burdet *et al* (1997), rhGH was used in an attempt to increase muscle mass and muscle strength. While these interventions were successful at increasing muscle mass, there was no change in muscle strength.

It has also been reported that there is reduced muscle endurance in this patient group (Serres *et al*, 1998). Serres *et al* (1998) reported that COPD patients achieved fewer dynamic contractions of the quadriceps when matched for maximal strength than controls. In addition to the observed changes in muscle strength following Cr loading, a significantly greater increase in muscle endurance was also observed (as measured by total work and total repetitions) in the Cr group compared to the placebo group. This finding is in agreement with previously

published data with respect to observed increases in muscle endurance in healthy resistance-trained humans (Experiment 1), healthy older subjects (Rawson *et al*, 1999; Gotshalk *et al*, 2002) and heart failure patients (Gordan *et al*, 1995; Andrews *et al*, 1998).

The exact mechanisms behind these observed increases are still not clear. As muscle biopsies were not obtained in this study, one can only speculate on the potential mechanisms for this improvement in muscle strength and muscle endurance following Cr supplementation. However it has also been shown that patients with COPD have peripheral muscle abnormalities. For example studies by Gertz *et al* (1977) and Jakobsson *et al* (1990) have demonstrated lower concentrations of ATP and PCr at rest using muscle biopsies, and other studies have also shown lower intracellular pH and slower PCr resynthesis during recovery from exercise (Payen *et al*, 1993; Sala *et al*, 1999). Two of the possible mechanisms behind the ergogenic effect of Cr supplementation are its ability to increase resting levels of PCr and also increase the rate of PCr resynthesis following intense exercise. Therefore, theoretically Cr supplementation should be able to rectify these abnormalities in skeletal muscle, and hence increase muscle endurance and muscle strength. In addition, because fatigue during the types of tests (maximal and strength endurance) utilized in the present study have previously been attributed to depletion of muscle PCr stores (Hultman *et al*, 1990; Tesch *et al*, 1989), Cr supplementation has a greater potential to increase muscle

performance. A number of previous studies support this view (Balsom *et al*, 1995; Greenhaff *et al*, 1994; Experiment 1).

A second and very important component of the present study was to evaluate the effectiveness of Cr supplementation when combined with a standard pulmonary rehabilitation programme, compared to pulmonary rehabilitation alone. With respect to this aspect of the study, the Cr group had significantly greater increases in hand grip strength (dominant hand only) over the supplementation/rehabilitation and rehab-load periods compared to the placebo group. Upper and lower body endurance as measured by total repetitions and total work respectively was also significantly increased in both groups over the supplementation/rehabilitation and rehab-load periods, with the magnitude of the increase being significantly greater in the Cr group compared to the placebo group. These results may indicate that Cr acted as an extra stimulus during training allowing patients in the Cr group to train at a greater intensity and thus improve their muscle strength a greater degree. Unfortunately, in the present study there was no measure of work completed per training session, however the significantly greater loss of FM observed in the Cr group would suggest this.

The physiological basis for a possible ergogenic effect of Cr supplementation on strength training is primarily two fold. Firstly, Cr supplementation has been shown to increase the number of repetitions performed per set (Earnest *et al*,

1995; Volek *et al*, 1999). Secondly, Cr supplementation has been shown to increase the rate of Cr rephosphorylation during the second minute of recovery from intense intermittent-type exercise (Greenhaff *et al*, 1994). Theoretically, both these physiological changes would allow an individual to train at a greater intensity compared to training without the use of this putative ergogenic aid. The enhanced muscle performance seen in the present study when combined to training (pulmonary rehabilitation) has also been observed in both healthy young subjects (Vandenberghe *et al*, 1996; Kreider *et al*, 1998; Volek *et al*, 1999) and healthy older subjects (Chrusch *et al*, 2001). The present study is the first to examine the effectiveness of Cr supplementation combined with a training stimulus in a patient population.

As already mentioned, following Cr loading, patients entered into a standard pulmonary rehabilitation programme. However during this programme a number of patients dropped out due to an increase in exacerbations or inability to cope with the training sessions. It is worth noting that the majority of the patients who dropped out were in the placebo group, with only one dropout during the programme in the Cr group (two others dropped out at the very end, but had completed the training). The reason for this is not clear however it could be linked to the already mentioned increase in body mass and/or an increased ability to cope with the training demands.

CONCLUSION

In conclusion, although this present study demonstrates some very positive outcomes with regard to the ergogenic effects of Cr in COPD patients (both short- and long-term), it still remains to be determined whether or not the observed benefits in the present study will have a positive impact on the daily activities and, more importantly, the quality of life of this patient population.

CHAPTER SEVEN

General Discussion

GENERAL DISCUSSION

The primary objectives of all four Experiments were to determine the effects of Cr supplementation on body composition and exercise performance in subjects of varying training and health status.

The aim of Experiment 1 was to determine the effects of Cr supplementation on body composition, muscle strength and muscle endurance in a group of highly resistance-trained male subjects. The results of this study suggested that 20 g Cr·d⁻¹ for 5 d resulted in an increase in peak force and total work during repeated isometric bench-press contractions but only when "non-responders" were removed. A negative correlation between estimated muscle Cr uptake and training status (Figure 3.4a) and also a positive correlation between estimated muscle Cr uptake and increase in exercise performance (Figure 3.4c) were also observed in Experiment 1. An explanation for the demonstration of "responders" and "non-responders" to creatine supplementation was therefore sought in Experiment 2. This second study was designed to optimise muscle Cr uptake during Cr supplementation using strategies previously shown in the literature (e.g. exercise, carbohydrate ingestion) to enhance muscle Cr uptake, using a group of non-resistance trained subjects. As was the case for Experiment 1, Experiment 2 also showed large inter-individual response to Cr supplementation, with a performance benefit being only seen once the Cr group

was subdivided into "responders" and "non-responders" (as in Experiment 1). These two studies argue strongly for the issue of "responders" and "non-responders" being multifactorial in nature.

A further outcome of Experiments 1 and 2 was that Cr supplementation stimulated an increase in body mass. Experiment 2, further, was able to ascribe this (in part, at least) to increases in total body water (TBW) and intracellular water (ICW). Experiment 3 was therefore designed to determine the effects of these Cr-stimulated increases in body water compartments on the ability of trained endurance cyclists to perform cycling exercise to exhaustion during heat exposure. The results from this study argue for a possible role for Cr supplementation for prolonging time to exhaustion (TTE) during exercise in the heat. Again, however, these results only became clear once the Cr group was divided into "responders" and "non-responders".

Finally, Experiment 4 examined whether the increases observed in Experiments 1 and 2 with regard to body composition, muscle strength and muscle endurance in healthy subjects could lead to similar increases in patients with moderate to severe Chronic Obstructive Pulmonary Disease (COPD). The outcome of the study was that Cr loading lead to significantly greater increases in body mass, fat-free mass, lower body muscle strength, upper and lower body muscle endurance compared to the placebo group. Following Cr and rehabilitation the

increases observed with regard to muscle strength and muscle endurance was significantly greater than those observed in the placebo group.

The major findings from these several studies are discussed collectively in this chapter and, as appropriate, conclusions made regarding the factors that may limit exercise performance in health and disease.

Cr Supplementation and Muscle Cr Uptake ("responders" and "non-responders")

As previously mentioned, the results of the first three experiments demonstrated that Cr supplementation was effective at increasing exercise performance in healthy young subjects, but only for those in whom muscle total [Cr] ([TCr]) was significantly increased following supplementation ("responders"). The concept of "responders" and "non-responders" was first proposed by Greenhaff *et al* (1994) and subsequently by Casey *et al* (1996). Implicit in the classification of subjects into "responders" and "non-responders" (Greenhaff *et al*, 1994; Casey *et al*, 1996) is the existence of what has been termed an "ergogenic threshold" for Cr uptake, which has been demonstrated to occur at about 20 mmol·kg⁻¹·dry muscle weight (Greenhaff *et al*, 1994 and Casey *et al*, 1999). The rationale for this assertion was the demonstration by Greenhaff *et al* (1994) that an increased rate of PCr resynthesis during recovery from exercise following Cr ingestion was evident in subjects whose muscle Cr concentration

was increased by on average 20 mmol·kg⁻¹·dry muscle weight, but not in those whose muscle Cr concentration increased by < 10 mmol·kg⁻¹·dry muscle weight. Our findings provide support for this contention. However, we were not able to assign a specific threshold value as intramuscular Cr uptake was only estimated. Two very distinct groups were evident in all three Experiments (1 - 3), giving support to the proposed "ergogenic threshold". The estimated Cr uptakes in all three Experiments were similar to those measured by Greenhaff *et al* (1994).

However although Greenhaff *et al* (1994) and Casey *et al* (1996) proposed the existence of subpopulations of "responders" and "non-responders", the present set of Experiments (1 - 3) were the first to further subdivide the Cr group into "responders" and "non-responders" based on estimated or measured muscle Cr uptake following Cr supplementation and to reanalyse the data based only on the "responders" data only. Therefore it is perhaps not surprising that, despite many Cr supplementation studies showing an ergogenic effect on exercise performance (Harris *et al*, 1992; Greenhaff *et al*, 1993; Soderlund *et al*, 1994; Balsom *et al*, 1993; Balsom *et al*, 1995; Greenhaff *et al*, 1994; Casey *et al*, 1996; Green *et al*, 1996; Earnest *et al*, 1995), there are still a significant number of studies which have not reported an ergogenic effect (e.g. Cooke *et al*, 1995; Odland *et al*, 1997; Snow *et al*, 1998; McKenna *et al*, 1999; Gilliam *et al*, 2000; Deutekom *et al*, 2000; Finn *et al*, 2001). It is tempting to speculate that this lack of effect (Cooke *et al*, 1995; Odland *et al*, 1997; Snow *et al*, 1998; McKenna *et al*, 1999; Gilliam *et al*,

2000; Deutekom *et al*, 2000; Finn *et al*, 2001) may reflect, in part at least, there having been no differentiation of subjects into "responders" and "non-responders" on the basis of measured or estimated muscle Cr uptake. That is, many studies failed to sample skeletal muscle changes in [TCr], either directly or indirectly (Cooke *et al*, 1995; Odland *et al*, 1997; Gilliam *et al*, 2000; Deutekom *et al*, 2000), and thus verify the efficacy of their supplementation protocols. This represents an important omission, as the potential ergogenicity of Cr supplementation has been shown to be dependent on the magnitude of the [TCr] increase following Cr supplementation (Casey *et al*, 1996, Snow *et al*, 1998). This point is supported by the demonstration in Experiments 1 and 2 (Figure 3.4c, Figure 4.5b-d) of positive correlations between estimated muscle Cr uptake and improvement in exercise performance.

Two recent studies further highlight this point. Snow *et al* (1998) and McKenna *et al* (1999) both examined the effects of short-term Cr supplementation (30g Cr d⁻¹ for 5 days) on 20 s maximal sprint and five 10 s maximal sprints, respectively. Neither study could demonstrate any additional effects of Cr loading on any of the variables measured, including body mass. However, closer inspection of the results of these studies do reveal that Cr supplementation did induce modest increases in [TCr]: 11.7 ± 2.4 mmol·kg⁻¹·dry muscle weight in the case of Snow *et al* (1998), and 23 mmol·kg⁻¹·dry muscle weight for McKenna *et al* (1998). Furthermore, the inter-individual responses showed a wide variability: a range

of 2.9 - 19.9 mmol·kg⁻¹·dry muscle weight for Snow *et al* (1998), and 7 - 40 mmol·kg⁻¹·dry muscle weight for McKenna *et al* (1999). One wonders what the outcomes of these two studies might have been if the researchers had subdivided their Cr group into "responders" and "non-responders". A further limitation of these studies is the small sample size (n= 8, Snow *et al*, 1998 and n=7, McKenna *et al*, 1999) which, coupled with lack of consideration of "responders" and "non-responders", could have increased the chance of both studies producing a Type II error.

Cr Loading and Muscle Strength and Muscle Endurance

As previously mentioned, not all studies in the literature have reported an ergogenic effect following Cr supplementation, with many of these negative results potentially explicable by relatively small increases in muscle Cr uptake following supplementation. However, 40 out of the 55 pertinent published studies on this topic have reported an ergogenic effect following Cr supplementation. Experiments 1, 2 and 4 examined the effects of Cr loading on muscle strength and endurance. In Experiments 1 and 2 Cr supplementation led to a significant increase in muscle strength and endurance (Figure 3.3 and Figure 4.4). However, the results of these studies only became clear when subjects were divided into "responders" and "non-responders" based on their estimated muscle Cr uptake. This finding was further supported by the positive correlations

observed in both Experiments 1 and 2 between estimated muscle Cr uptake and improvement in exercise performance (Figure 3.4c & Figure 4.5b-d, respectively), and Experiment 3 between estimated muscle Cr uptake and delta TTE ($r=0.75$, $n=11$, $P=0.008$), showing again that performance benefits are highly dependent on muscle Cr uptake. The results from Experiments 1 and 2 thus support the work of previous researchers with regard to the ergogenic effect of Cr supplementation on muscle strength and muscle endurance (Casey *et al*, 1996; Green *et al*, 1996, Kreider *et al*, 1998; Maganaris & Maughan, 1998; Volek *et al*, 1997).

In Experiment 4, the effects of Cr supplementation on body composition, muscle strength and endurance were examined in patients with moderate to severe COPD. Previously researchers have used controlled trials using such strategies as anabolic steroids (Yeh *et al*, 2002) and rhGH (Burnet *et al*, 1997) in an attempt to increase muscle mass and strength, while both these strategies have demonstrated improvements in muscle mass, no discernible effect on muscle function were demonstrated. Although researchers have previously examined the effect of Cr supplementation on muscle strength and muscle endurance in healthy older subjects (Rawson *et al*, 1999; Gotshalk *et al*, 2002) and in heart failure patients (Gordan *et al*, 1995; Andrews *et al*, 1998), Experiment 4 is the first study to examine the role of Cr supplementation in this patient population (COPD). Furthermore, the positive results obtained from Experiment 4 with

regard to muscle strength and endurance is, to our knowledge, the first showing positive effects on muscle strength and endurance of this patient group. However, the extent to which patients varied in their capacity to take up Cr is unclear at present, as we were not in a position to estimate Cr uptake in our patients. The important issue raised by this is that the potential for rehabilitation and reduced mortality in COPD may be influenced by intramuscular Cr uptake characteristics.

As muscle biopsies were not obtained in any of the present studies, one can only speculate on the potential mechanisms for the demonstrated improvement in exercise performance following Cr supplementation. We speculate that increased intramuscular PCr availability and PCr resynthesis during recovery from maximal exercise of the type employed in Experiments 1, 2 and 4 is perhaps the most plausible explanation, given that muscle fatigue has previously been associated with a depletion of muscle PCr stores (Hultman *et al*, 1990; Tesch *et al*, 1989). Cr supplementation has the potential to increase the basal levels of PCr and, would therefore be expected to better maintain the required ATP resynthesis rates during exercise (Harris *et al*, 1992) which, in turn, could lead to delayed onset of muscle fatigue. Also, the accelerated rate of PCr resynthesis during recovery from intense muscle contractions (Greenhaff *et al*, 1994) could consequently lead to an increased ability to rephosphorylate ADP. This suggestion by Greenhaff *et al* (1994) was further supported by their finding

of lower plasma ammonia accumulation during the repeated bouts of exercise following Cr ingestion, despite the Cr group producing higher work output.

Effects of Cr supplementation on body composition

The results of all 4 experiments demonstrate that short-term (5 - 14 d) Cr supplementation (15 - 20 g Cr d⁻¹) can lead to significant increases in body mass (Figure 3.2, Figure 4.2a, Figure 5.2 and Table 6.2). This finding has also been consistently observed throughout the Cr literature (Greenhaff *et al*, 1994; Earnest *et al*, 1995; Cooke *et al*, 1995, Green *et al*, 1996; Terrillion *et al*, 1997; Maganaris & Maughan, 1998; Krieder *et al*, 1998; Becque *et al*, 2000; Finn *et al*, 2001). Although the results of all 4 of the present studies support the theory that Cr supplementation is effective at increasing body mass under the conditions of the present studies, the exact mechanism behind this increase still remains unclear. To date, there have been two mechanisms proposed for this Cr-stimulated increase in body mass. It has been suggested that the increase in body mass following Cr supplementation was due to an increase in water retention (Hultman *et al*, 1996) which could, in turn, result in cell swelling and an increase in protein synthesis (Haussinger *et al*, 1993). Others, however, have attributed the increase in body mass following Cr supplementation to an increase in protein synthesis and the associated increase in water content (Kreider *et al*, 1998). The balance of available evidence from human performance studies using Cr

supplementation and more-direct evidence from animal *in vivo* and *in vitro* experiments supports the notion that increasing Cr availability may indeed increase protein synthesis (Ingwall *et al*, 1974, Kreider *et al*, 1998, Volek *et al*, 1999). However, not all studies have found significant differences in body mass following short-term Cr supplementation (Grindstaff *et al*, 1997, Steenge *et al*, 1998, Terrillion *et al*, 1997, McKenna *et al*, 1999; Snow *et al*, 1998). These negative findings could reflect negligible increases in [TCr] following Cr supplementation, with increases in body mass therefore not being expected. Taken together, the results of Experiments 1 - 3 demonstrated a positive correlation between estimated muscle Cr uptake and increase in body mass ($r=0.499$, $n=41$, $P=0.001$) (Figure 3.4b, Figure 4.5a) provides further support for the proposal that increases in body mass may provide an indirect measure of muscle Cr uptake.

As already mentioned, Cr loading increased body mass in all four of the present Experiments with subjects varying in training status and background, ranging from highly resistance trained individuals (Experiment 1), non-resistance trained individuals (Experiment 2), endurance trained subjects (Experiment 3) to patients with moderate to severe COPD (Experiment 4). The observed increases in body mass were similar between all three groups studied in Experiments 1 - 3, showing Cr to be effective at increasing body mass these particular populations, and were of similar magnitude to those previously reported (Greenhaff *et al*, 1994; Earnest *et al*, 1995; Cooke *et al*, 1995, Green *et al*, 1996; Terrillion *et al*, 1997;

Maganaris & Maughan, 1998; Krieder *et al*, 1998; Volek *et al*, 1999; Becque *et al*, 2000; Finn *et al*, 2001).

In Experiment 4, there was also a significant increase of body mass following short-term Cr supplementation in the group of patients with moderate to severe COPD. The importance of this finding can be seen from a recent study by Schols *et al* (1998). These authors demonstrated that COPD patients whose body weight increased significantly following administration of anabolic steroids (> 2kg/8 wks) had a decreased mortality risk compared to patients whose body weight did not increase following the intervention. For this reason, the observed increases in body mass following Cr supplementation during Experiment 4 are of particular clinical importance.

Cr supplementation combined with resistance training

Experiments 2 and 4 examined the effects of Cr supplementation combined with resistance training (Experiment 2) and a standard pulmonary rehabilitation programme (Experiment 4) compared to subjects training combined with placebo. While no additional increase in exercise performance was observed during Experiment 2 (possibly due to training status of the subjects and study duration), Cr supplementation led to significantly greater increase in muscle strength and endurance in patients with moderate to severe COPD. As

previously mentioned, many researchers have examined strategies for improving muscle function during pulmonary rehabilitation in patients with COPD. However, this present study is the first study to best of our knowledge to show a positive effect on muscle function in addition to the increases observed following pulmonary rehabilitation alone. The physiological basis for this ergogenic effect of Cr supplementation when combined with pulmonary rehabilitation is primarily two-fold. Firstly, Cr supplementation has been shown to increase the number of repetitions performed per set (Earnest *et al*, 1995; Volek *et al*, 1997). Secondly, Cr supplementation has been shown to increase the rate of PCr rephosphorylation during the second minute of recovery from intense intermittent exercise (Greenhaff *et al*, 1994). Theoretically, both of these physiological changes could allow patients to train at a greater intensity than without this putative ergogenic aid. During the pulmonary rehabilitation programme in Experiment 4, a number of patients dropped out due to an increase in exacerbations or an inability to cope with the training sessions. Interestingly, the majority of the patients who dropped out were in the placebo group, with only one dropout from the Cr group. The reason behind this is unclear, but could be linked to the already-mentioned increase in body mass and/or an increased ability to cope with the training demands. If it can be shown convincingly that Cr supplementation increases the compliance of patients to rehabilitation programmes, this would represent a very important effect of Cr supplementation in the patient population.

Statistical Limitations of Previous Studies

As previously mentioned, one possible explanation for the conflicting results in the literature with regard to the ergogenic potential of Cr supplementation has been the failure of many researchers to identify "non-responders" within their Cr group.

Recently, another possible explanation has been offered for these conflicting findings. Thus, Gilliam *et al* (1999) questioned the statistical techniques employed by many researchers in the "Creatine supplementation" field. They suggested that the multiple t-tests used by researchers such as Greenhaff *et al* (1993), Birch *et al* (1994) and Harris *et al* (1992) inflated the chance of producing a Type I error, and they therefore suggested that researchers in the future should use ANOVA (Gilliam *et al*, 1999). They also suggested this as a possible reason behind many of the conflicting findings with regard to the ergogenic potential of Cr supplementation. Studies that have previously used ANOVA's and found negative effects include these of Burke *et al* (1996), Odland *et al* (1997) and Redondo *et al* (1996). In all four experiments completed in this thesis, pre-planned statistical analysis was carried out (ANOVA). Furthermore, sensitivity was improved by expressing the responses not simply as absolute values but also as "post-pre" (delta). The use of delta takes into consideration any "placebo" effect that might be observed in the placebo group, therefore reducing the chance of a

Type I error. Another analytical consideration that may contribute to the contradictory findings of many previous studies is the small subject number used by many studies in the Cr literature. A small sample size, combined with the possibility of the Cr group comprising of "non-responders" could increase the chance of many studies producing a Type II error. These shortcomings were taken account of in the design of the present studies: the relatively large sample sizes and the identification of the "non-responders" within the Cr group help prevent the present Experiments for making a Type II error (Tarnopolsky & MacLennan, 2000).

Directions for Future Research

The results from Experiments 1 - 3 only became apparent when the Cr group was subdivided into "responders" and "non-responders" based on their estimated muscle Cr uptake and, as already mentioned, this is one of the possible factors behind the conflicting results obtained within the literature with regard to the ergogenic potential of Cr supplementation. Therefore researchers should try and further identify factors contributing to the "responders" and "non-responders" issue, such as the mechanisms behind intramuscular Cr uptake, and the adaptive potential of these mechanisms to different forms of training and exercise-related dietary regimens. It is important to point out, also, that there is uncertainty as to whether an intramuscular [Cr] in the region of $20 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{dry}$

muscle weight represents the actual threshold value (Greenhaff et al., 1994) or whether more detailed investigations might show this to be somewhat lower. Furthermore, the evidence for there being an actual threshold characteristic rather than a simple proportional graded relationship between intramuscular [Cr] and PCr resynthesis capacity has not convincingly been presented to date. In addition, future studies could attempt to eliminate the "responders" issue a priori. For example, researchers could supplement their subjects for 2-3 days with Cr supplementation and based on their individual responses separate their subjects into "responders" and "non-responders". Then after the 4-6 wk washout period include only the "responders" to Cr supplementation in their study.

Experiment 4 was the first intervention study to show positive effects on muscle strength and muscle endurance in patients with COPD. However, additional more comprehensive experiments are needed to resolve the extent to which Cr supplementation may facilitate the rehabilitation and mortality of this patient group. Although the overall effect on body mass, muscle strength and muscle endurance were positive, we were not in a position to assess how quality of life was affected. A further, important observation that requires more attention is whether Cr supplementation in this patient group does indeed influence compliance with pulmonary rehabilitation and, if so, the extent to which this is a consequence of an increase in body mass or an increased ability to cope with the

training demands. Finally, the extent to which the disease process in COPD may influence Cr transport capacity also requires attention.

GENERAL CONCLUSIONS

From this series of experiments a number of conclusions can be made. These conclusions apply specifically to the protocols applied in the present set of experiments.

- From the results of all 4 experiments it can be concluded that short-term Cr supplementation significantly affects body mass and body composition, although the exact mechanism behind these observed increases are still under debate.
- From the results of experiments 1 and 4 it can be concluded that short-term Cr supplementation is effective at increasing muscle strength and endurance probably due to increased resting [PCr] and/or increased rates of PCr resynthesis.
- From the results of experiment 4 it can be concluded that Cr supplementation was an effective strategy for increasing muscle strength and endurance to a significantly greater extent when combined with a pulmonary rehabilitation program than pulmonary rehabilitation alone.

- From the positive correlations found in experiments 1 -3 between estimated muscle Cr uptake and performance it can be concluded that the performance benefit obtained from Cr supplementation is very dependant on initial [PCr] and the level to which these are increased following Cr supplementation.
- From the results on experiment 3 it can be concluded that exercise in the heat is not adversely affected by Cr supplementation and that the preliminary results on this experiment suggest a potential role for Cr supplementation during exercise in the heat, although much research is still required.

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APPENDIX

Consent Form



UNIVERSITY
of
GLASGOW

CONSENT FORM

TITLE OF PROPOSED STUDY

Name of volunteer:

Principle Investigator: **Dr. Y.P. Pitsiladis**

I have read the volunteer information sheet on the above mentioned study and have had the opportunity to discuss the details and ask questions. The nature and purpose of the tests to be undertaken have been explained to me. I understand fully what is proposed to be done.

I have agreed to take part in the study as it has been outlined to me, but I understand that I am completely free to withdraw from the study at any time I wish.

I understand that these trials are part of a research project designed to promote medical knowledge, which has been approved by the Joint Ethical Committee, and may be of no benefit to me personally.

I hereby fully and freely consent to take part in the study which has been explained to me.

Signature of volunteer

Date

I confirm that I have fully explained to the volunteer named above, the nature and purpose of the tests to be undertaken.

Signature of investigator

Date