THE EFFECTS OF CREATINE SUPPLEMENTATION ON PERFORMANCE AND METABOLISM DURING BRIEF, INTERMITTENT, HIGH-INTENSITY EXERCISE

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Master of Applied Science

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October, 1996

DECLARATION

This dissertation is the result of original, previously unpublished work conducted at Victoria University of Technology, Footscray, in the Department of Human Movement, Recreation and Performance. The work was performed by the author, with some assistance provided by others, as acknowledged on page iii.

Chu Ba Binh

October, 1996

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my research supervisors, Dr. Steve Selig and Dr. Michael McKenna, for their time and effort as well as advice and friendship throughout the study program. Without their support, the completion of this dissertation would have been much more difficult.

I would also like to thank Prof. David Lawson for his continued assistance, encouragement and support for this project. Thanks to Rod Snow for reading a draft of the thesis and assistance with biochemistry.

I need to thank many people involved in the data collection phase of the study, in particular, Termboon Sangkabutra, Simon Sostaric and Justin Kemp, a "crazy man," who helped a lot with recruitment of subjects.

Thanks to the technical staff of the department, in particular, Travis Newing, Ian Fairweather, Danny Rutar and Michael Manders, for their ongoing technical assistance. Thanks to Mr. Harry Gielewski and Ms. Berchan Erthberth, Department of Computer and Mathematical Sciences for consulting in statistics.

To the 14 subjects, a big thank you for the commitment to the project and enthusiasm during data collection.

To my family in VietNam, thanks for being patient and supporting me in many ways. I am proud of you mum! To my friends and all others, I would specially like to thank for their support and encouragement, in particular Ron and Christine for understanding and sharing.

Finally, I dedicate this thesis to Huong T. T. Nguyen, whose love gave me motivation to study. "I will steal you with the fence!"

ABSTRACT

This study investigated the ergogenic and metabolic effects of creatine supplementation during two series of 10 x 6 s of "all out" cycling sprints, interrupted by either 30 s or 6 min rest intervals (RI). Both sprint series were performed before and after five days of oral supplementation with either creatine (CrS, n = 7) or placebo (CON, n = 7), using a randomised double-blind design. To evaluate the effects of CrS on exercise performance, peak power (PPO) and mean power (MPO) were determined during the 30 s and 6 min RI sprint series. To monitor the metabolic effects of CrS, arterialised venous blood was sampled at rest, immediately after the fifth and tenth sprints and during 20 min of recovery; these were analysed for plasma ammonia, lactate and hydrogen ion concentrations.

PPO was maintained at near constant levels for 6 min RI for both supplementation groups, but decreased progressively for 30 s RI, regardless of supplementation. Following CrS, PPO and MPO increased ($P < 0.05$) during both RI trials, but not with CON. Plasma ammonia concentrations ($[NH_3^+]$) were significantly lower after CrS for 6 min RI, and tended to be lower for 30 s RI. For CON, $[NH_3^+]$ was not significantly different after supplementation. Plasma lactate and hydrogen ion concentrations were similar before and after CrS for both RI, despite the higher power after CrS. The corresponding responses for CON were similar to CrS, with the exception that $[La^-]$ was significantly lower after supplementation for 30 s RI.

Oral creatine supplementation enhanced performance during repeated "all out" sprints of short duration, for a wide range of rest intervals between sprints. The ergogenic effects of CrS were probably associated with decreased degradation of adenine nucleotides, reflected by lower plasma $[NH_3^+]$, and greater ATP turnover, caused by an increase pre-exercise PCr availability.

PUBLICATIONS

The following publications are related to this dissertation:

1. The effects of creatine supplementation on performance of high-intensity intermittent exercise. Abstract. *7th Annual Victorian Conference on Student Research. Exercise Science, Sports Administration, Physical Education and Sport.* Deakin University. Melbourne, 6 October, 1995.

2. The effects of creatine supplementation on performance of high-intensity intermittent exercise. Abstract. *3rd Annual Conference*. Faculty of Human Development Victoria University of Technology, Melbourne, 11-14 July, 1996.

3. The effects of creatine supplementation on performance of high-intensity intermittent exercise. Abstract. *1st International Annual Conference on Exercise Science*. Griffith University, Gold Coast, Queensland, 11-14 July, 1996.

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CHAPTER 1 INTRODUCTION

Ergogenic aids are substances or devices that enhance exercise performance and are used by many athletes wishing to optimise performance. The enhancement of human performance by nutritional means has been studied and applied for many years (reviewed by Bucci, 1993). Many elite athletes are willing participants in informal, personal experimentation with ergogenic aids. Until this century, research into ergogenic aids was mainly confined to foodstuffs. Dietary manipulations are still widely used by many elite performers, because they are generally safe and legal (except excessive caffeine), and often effective (eg. carbohydrate loading). A nutritional supplement that has recently attracted much interest is creatine monohydrate (CrH_2O).

In its phosphorylated form (PCr), creatine has a function in cellular adenosine triphosphate (ATP) homeostasis (Watts 1975; Bessman and Geiger, 1981; Elington 1989; Harris et al., 1992). The most rapid method of producing ATP involves the donation of a phosphate and its bond energy from PCr to adenosine diphosphate (ADP) to form ATP (Fox et al., 1989; Hultman et al., 1990 Maughan 1995). The reaction is catalyzed by the enzyme creatine kinase (CK).

$$ATP + H_2O \xrightarrow{\text{ATP ase}} ADP + Pi + H^+ + \textbf{Energy}$$

$$PCr + ADP + H^+ \underset{\longleftarrow}{\xrightarrow{\text{Cr kinase}}} ATP + Cr$$

PCr is not only an immediate buffer for ATP resynthesis, but is also thought to facilitate energy translocation from mitochondria to sites of ATP utilisation (Bessman, 1985; Wallimann, et al., 1992). Muscle cells, however, store only a small, limited quantity of PCr (Karlsson et al., 1970; Maughan 1995). Since muscle PCr concentration can fall to almost zero during intense exercise (Hultman et al., 1967; Karlsson et al., 1970), the amount of ATP that can be formed via this reaction is limited. During maximal exercise, the availability of PCr has been implicated as a limiting factor for ATP turnover and as an important factor in attaining and maintaining muscle power (Hultman et al., 1967, 1991; Katz et al., 1986).

Dietary supplements of Cr are readily absorbed and when supplementation (CrS) is continued over a period of days, the total creatine pool (TCr) in muscle is increased (Crim et al., 1976; Harris et al., 1992; Greenhaff et al., 1994a; Balsom et al., 1995; Febbraio et al., 1995; Hultman et al., 1996). Of the increase in TCr, about 70% is in the form of free creatine, with the balance as PCr. The performance of brief, intermittent, high-intensity exercise (BIHIX) may be improved by CrS (Balsom et al., 1993a &1995; Greenhaff et al., 1993; Dawson et al., 1995).

Several inter-related metabolic mechanisms have been proposed to explain the ergogenic effects of CrS during intermittent high-intensity exercise (HIX). These include increases in TCr and PCr contents at rest (Harris et al., 1992; Febbraio et al., 1995; Balsom et al., 1995), PCr utilisation during exercise (Greenhaff et al., 1994a), PCr resynthesis during recovery (Greenhaff et al., 1994a) and muscle buffering capacity during exercise (Greenhaff et al., 1994a), and reductions in adenine

nucleotide degradation (Balsom et al., 1993a) and lactate production (Balsom et al., 1993a).

The influence of CrS on the performance of BIHIX has been actively researched in the last few years, but no prior studies have compared the effects of CrS for BIHIX using long versus short rest intervals (RI). This study examined the effects of CrS on exercise performance and metabolism of two series of 10 x 6 s maximal-intensity cycling sprints, one with 30 s RI, the other with 6 min RI which some sports might fit with (e.g. 100m, 200m, long jump, high jump). The 6 min RI was selected on the basis that it was long enough for almost complete PCr resynthesis (McCartney et al., 1986; Bogdanis et al., 1995, 1996), whilst PCr resynthesis was expected to be no more than 50% complete for the 30 s RI (Balsom et al., 1995). A practical application of the study was to describe the forms of intermittent exercise for which CrS is more effective.

CHAPTER 2 LITERATURE REVIEW

Section A reviews the literature on exercise metabolism during high-intensity exercise, focussing on intermittent exercise. Section B concerns the influence of CrS on performance and metabolism during brief, intermittent, high-intensity exercise (BIHIX).

Section A: Exercise Metabolism during High-Intensity Exercise

2.1 Overview of Exercise Metabolism

Energy derived from oxidation of food is not transferred directly to the muscle for biologic work. Rather, it is transferred to a compound with very high energy bonds, adenosine-5'-triphosphate (ATP), which is then utilised for all of the energy-requiring processes of the cells, and referred to as a "high-energy phosphate". ATP is broken down (hydrolysed) by enzymes collectively termed ATP-ases in a reaction which liberates approximately 7.3 kcal of energy per mole of ATP (McArdle, et al., 1991). ATP is the most important energy transporter in the cell, and without sufficient amounts of ATP most cells quickly die. However, only small amounts of ATP are stored in the cell, around 5-6 $mmol \cdot kg^{-1}$ of wet muscle tissue mass (Saltin and Gollnick, 1983). Metabolic pathways must exist in the cell with the capacity to rephosphorylated ATP, because muscular exercise requires an uninterrupted supply of ATP to provide the energy needed for cellular function, including contraction. Both aerobic and anaerobic energy systems contribute to ATP turnover during exercise (Hultman et al., 1991; Table 2.1). Under anaerobic conditions there are three resynthesising pathways contributing to the

continued supply of ATP in exercise: creatine phosphate hydrolysis, glycolysis and the catabolism of adenine nucleotides. Aerobic generation of ATP consists of metabolism of carbohydrates, fats and, to a lesser extent, protein.

Substrate (products)	Max. ATP formation rate (mmol. s^{-1}. kg^{-1} dry muscle)	Available store (mmol. kg^{-1} dry muscle)
ATP, PCr (ADP, Cr)	11.2	100
Glycogen (lactate)	6.0	~ 250 (or totally 1,030)
Glycogen (CO_2 + H_2O)	2.2-2.9	13,000
Fatty acids (CO_2 + H_2O)	1.0	Not limiting

Table 2.1: Maximal rates of ATP resynthesis from different substrates and available stores (reproduced from Hultman et al., 1991).

2.2 Anaerobic Metabolism

2.2.1 Creatine Phosphate Metabolism

PCr hydrolysis provides most of the immediate energy for the resynthesis of ATP during intense physical activity lasting for up to a few seconds. Two-to-three fold more cellular energy is stored in the form of PCr than ATP. In reaction (1) (below), PCr is cleaved by

the enzyme creatine-kinase (CK) and its phosphate is donated directly to ADP to resynthesise ATP; this reaction is reversible.

$$PCr + ADP + H^{+} \overset{CK}{\rightleftharpoons} ATP + Cr \quad (1)$$

In most cells the action of creatine-kinase is so rapid, it is difficult to determine an overall decrease in ATP level after a twitch or contraction; ie. ATP is replenished almost immediately by PCr. The hydrolysis reactions of ATP and PCr present the most rapidly available source of energy for use by skeletal muscle, because these reactions do not depend on: i) transport of oxygen to working muscle, ii) a cascade of chemical reactions and iii) PCr and some ATP are stored in proximity to the contractile mechanism of muscles. At very high-intensity exercise PCr concentration is rapidly depleted (Hultman et al., 1967), before being resynthesised during recovery (Hultman et al., 1967; Sahlin et al., 1979; Bogdanis et al., 1995).

The repletion of PCr during recovery is much slower than the rate of utilisation during exercise. Bogdanis et al. (1995) modelled the time course of PCr resynthesis following maximal exercise, demonstrating that PCr was resynthesised at an initial rapid rate and then at a more gradual rate. In their study, PCr content in active muscle had fallen to less than 20% of the resting level immediately following 30 seconds of maximal sprint cycling exercise, before recovering to about 65% of the pre-exercise concentration after 1.5 min of recovery, but reached only about 85% after 6 min (Bogdanis et al., 1995). Using a similar exercise protocol, McCartney et al. (1986) reported that PCr content had recovered to about 80% after 4 min. The slow PCr resynthesis after this type of exercise was suggested to be related to the high exercise intensity and to a reduced blood flow in the legs during recovery (Bogdanis et al., 1995). PCr resynthesis is inhibited if the local

circulation to muscle is occluded, and increased when the occlusive device is removed (Harris et al., 1976; Sahlin et al., 1979). Sahlin et al. (1979) proposed that the initial fast phase of PCr resynthesis is limited by the availability of oxygen whereas the subsequent slow phase is limited by hydrogen ion transport from the muscle.

It has been suggested that the availability of PCr is a limitation to ATP turnover during brief maximal exercise (Cheetham et al., 1986). Bogdanis et al. (1996) found a high correlation between PCr resynthesis during 4 min of recovery from a 30 s sprint and power during the initial 10 s of a second 30 s sprint ($r = 0.91$), but no such relationships were found during the last 20 s of the sprint. It was suggested that the availability of PCr before a repeated sprint may be related to the capacity to generate high power during the first few seconds of a sprint (Bogdanis et al., 1996).

The decrements in power during maximal intermittent exercise are more closely related to falls in PCr content than ATP content (McCartney et al., 1986; Gaitanos et al., 1993). McCartney et al. (1986) studied the responses to four 30-s bouts of maximal isokinetic cycling, interrupted by 4 min rest intervals (RI), and found that muscle ATP content decreased to 60% of the resting level after the first sprint and decreased further to 57% after the third but increased to 62% after the fourth sprint. At the same time PCr content had declined to 30% of rest immediately following the first sprint and to 12% and 4% immediately after the third and the fourth sprints, respectively.

More recently, Gaitanos et al. (1993) studied the responses to 10 x 6 s maximal cycling sprints with 30 s RI, reporting a 57% fall in PCr content after the first sprint, but only a 13% fall in ATP content. There was almost no further change in ATP content during the final sprint, while PCr content fell from 50% to 16% of resting levels. The authors

calculated an ATP production rate for the first sprint of 14.9 mmol. kg dry wt^{-1} s^{-1} (Table 2.2, below), estimated from the changes in the concentration of muscle metabolites, which was close to the theoretical maximal rate of 17 mmol· kg dry wt^{-1}·s^{-1} proposed by McGilvery (1975). During this sprint, PCr contributed about 50% of the ATP production rate, with the remainder being derived predominantly from glycolysis (44%). In the final 6 s sprint, ATP production was maintained by similar contributions from PCr hydrolysis and oxidative phosphorylation (Gaitanos et al., 1993). These results indicate that PCr continued to contribute markedly to ATP production during the latter sprints of a series of BIHIX and emphasises the importance of PCr replenishment during recovery periods in this form of exercise.

	ATP production *mmol· kg dry wt*		*ATP production rate* *mmol · kg dry wt^{-1} · s^{-1}*	
	Sprint 1 (n =8)	Sprint 10 (n = 7)	Sprint 1 (n =8)	Sprint 10 (n = 7)
Total	89.3	31.6	14.9	5.3
From glycolysis	39.4	5.1	6.6	0.9
From PCr	44.3	25.3	7.4	4.2

Table 2.2: Estimates of ATP production from anaerobic sources during the 1st and 10th of 6 s sprints of exercise (data reproduced from Gaitanos et al., 1993).

2.2.2 Adenine Nucleotide Metabolism

Adenylate kinase (AK) can supply ATP rapidly to the contractile system during high-intensity exercise (Sjödin, 1992; reaction (2), below).

$$ADP + ADP \xrightarrow{AK} ATP + AMP \qquad (2)$$

Adenylate kinase can exist in either the isozyme fractions closely associated with the actomyosin complex (Savabi et al., 1986) or the fractions located in the mitochondrial area (Bessman and Carpenter, 1985). During intense exercise, when the ADP concentration is elevated, the adenylate kinase reaction contributes to ATP resynthesis, albeit a small contribution. Boobis et al. (1987) suggested that during brief high-intensity exercise, glycolysis and PCr degradation contributed almost entirely to the resynthesis of ATP with the remaining minor fraction (~4%) likely to be coming from the adenylate kinase reaction. This suggestion was supported by Gaitanos et al. (1993), who calculated that glycolysis and PCr contributed about 94% of the total ATP production from anaerobic sources during the first of ten 6 s sprints, with the remainder attributed to adenine nucleotide degradation. Although offering only a small contribution to total ATP production, the importance of the pathway for athletes was proposed by Thorstensson et al. (1975), who found an increase in the activity of adenylate kinase following 8 weeks of training comprising repeated 5 s sprints. In a recent study, Stathis et al. (1994) reported that ATP and the total adenine nucleotide pool at rest were reduced by 19% and 18% respectively, after 7 weeks of training using 30 s "all-out" sprints.

During high-intensity exercise, the myofibrillar fraction of PCr may drop very rapidly (Boobis et al., 1982, Sjödin, 1992), leading to accumulation of ADP, H^+, and inorganic phosphate (Pi) (Sjödin, 1992). These metabolic conditions favour the formation of ATP

and AMP from ADP via the adenylate kinase reaction (reaction No.(2) & Fig. 2.1). AMP can be degraded through two possible pathways (Fig. 2.1): via AMP deaminase (reaction (3), below) or dephosphorylation to adenosine (Sahlin and Katz, 1988). Some studies have indicated that increases in IMP and ammonia are often accompanied by a proportional decline in muscle ATP content during intense exercise in humans (Katz et al., 1986b; Graham et al., 1990; Stathis et al., 1994). However, other studies have observed the relationship between ATP degradation and IMP formation or ATP degradation and [NH_3^+] accumulation is not always matched (Bangsbo et al., 1992; Febbraio et al., 1996). AMP degradation is via its deamination during this type of exercise.

$$AMP + H_2O \rightarrow IMP + NH_3^+ \qquad (3)$$

There are two metabolic fates of IMP: reamination to AMP by the enzymes adenylosuccinate synthetase and adenylosuccinate lyase, or dephosphorylation to inosine by the enzyme 5'-nucleotidase (Fig. 2.1; Lowenstein 1972; Sahlin et al., 1990). The final product of AMP oxidation within the cytosol of the muscle cells is hypoxanthine (Hellsten-Westing et al., 1991; Sjödin, 1992), which can diffuse through the sarcolemma to the interstitial space and through capillary endothelial cells into the bloodstream. Hypoxanthine can be oxidized to uric acid in capillary endothelial cells via the action of xanthine oxidase. Alternatively, hypoxanthine may be salvaged back to the muscle adenine nucleotide pool (Fig. 2.1). The transformation to uric acid is an irreversible reaction (Hellsten -Westing et al., 1991; Sjödin 1992).

Plasma concentrations of hypoxanthine and uric acid have been reported to increase in response to maximal-intensity intermittent exercise (Balsom et al., 1992a & b). The

formation of these purines during exercise indicates a net degradation of ATP in muscle (Fig. 2.1). The production of uric acid was suggested as evidence of a partial depletion of the adenine nucleotide pool (Balsom et al., 1992a & b).

a) Plasma Ammonia

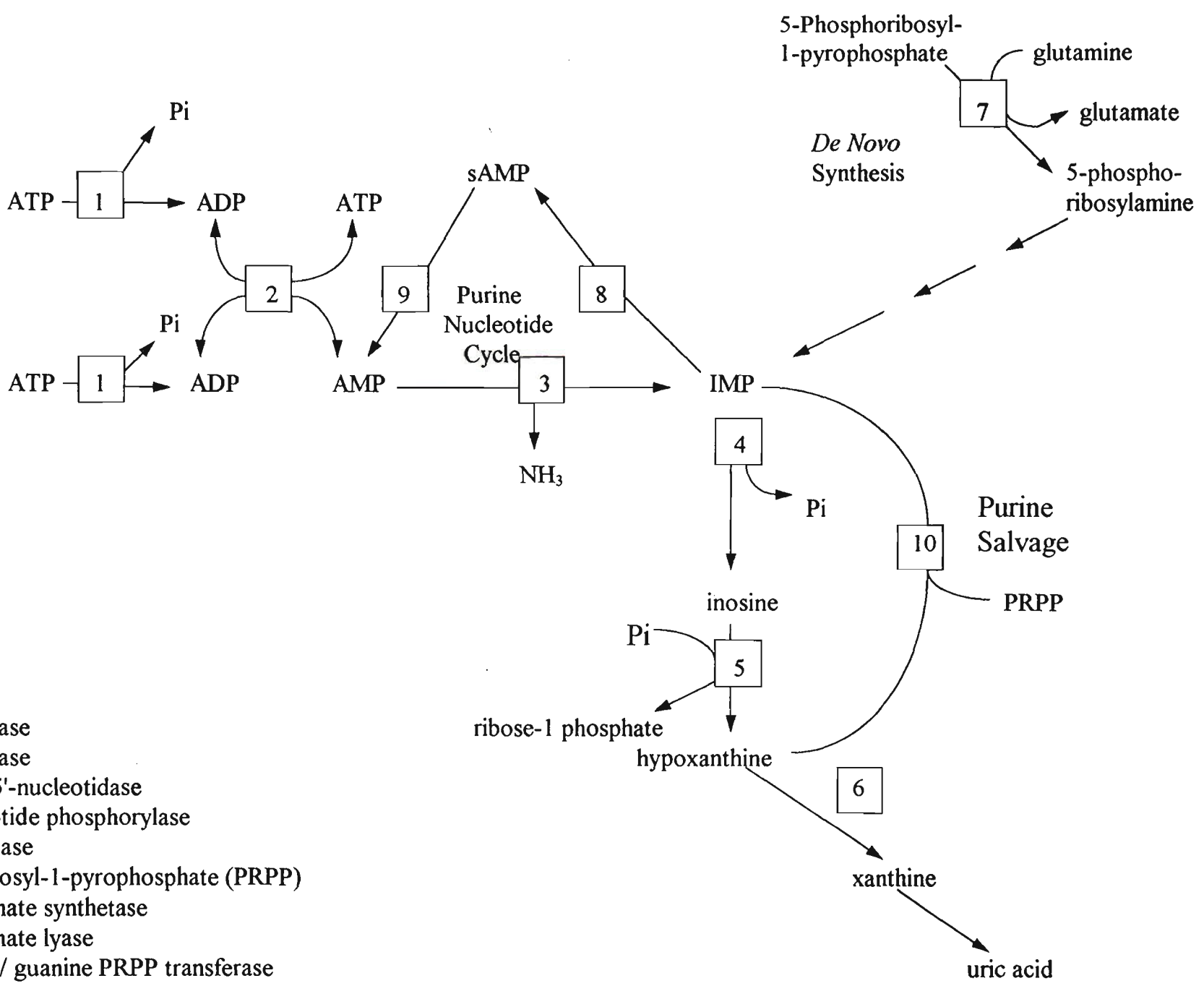
The baseline concentrations of ammonia in healthy individuals ranges between 5-45 μM in venous plasma (Svensson and Änfalt, 1982; Mineo et al., 1985; Snow et al., 1992) and 40-85 μM in venous whole blood (Babij et al., 1983., Dudley et al., 1983., Wilkerson et al., 1977). During high-intensity exercise, adenine nucleotide degradation in skeletal muscle is the major source of ammonia in blood (Ericksson et al., 1985; Graham et al., 1990), with less appearance from other tissues, such as kidneys and brain (Onstad et al., 1979; Sahlin & Katz 1988). Skeletal muscle ammonia may also be produced as a result of amino acid catabolism, but this pathway is relatively unimportant for BIHIX (Graham et al., 1990; Stathis et al., 1994).

Following high-intensity exercise, plasma ammonia concentrations are increased (Katz et al., 1986 a & b; Stathis et al., 1994), reaching a peak 8-10 fold higher than resting levels at 2 min recovery following a 30 s "all out" sprint (Graham et al., 1990; Snow et al., 1992). The extent of the rise in plasma ammonia concentration following high-intensity exercise depends on both the duration and intensity of exercise (Babij et al., 1983, Graham et al., 1987, Sahlin et al., 1990; Stathis et al., 1994). Furthermore, these levels are influenced by the training status of the individual (Snow et al., 1992; Stathis et al., 1994). In sprint-trained individuals, plasma ammonia levels were higher at 2 min of recovery from intense exercise, but were lower at 20 min, than before training (Stathis et al., 1994).

b) Plasma Hypoxanthine and Uric acid

Hypoxanthine is the substrate for the enzyme xanthine dehydrogenase which catalyses the production of uric acid from hypoxanthine (Fig. 2.1). While adenine nucleotides may be salvaged from hypoxanthine in a reaction catalysed by hypoxanthine-phosphoribosyltransferase, uric acid is produced in an irreversible reaction resulting in a loss to the total adenine nucleotide pool (Hellsten-Westing et al., 1991).

Following repeated bouts of maximal sprints, post-exercise plasma hypoxanthine and uric acid concentrations were higher when the sprint durations were longer for a given RI (ie higher work:rest ratio) (Balsom et al. (1992a). Similarly, post-exercise plasma hypoxanthine concentration was higher when a shorter RI was applied for a constant sprint distance (Balsom et al., 1992b); again the work:rest ratio was higher in this protocol. However in the latter study, there were no significant differences in the post-exercise concentrations of plasma uric acid for the different RI's. A possible explanation of this is that post-exercise hypoxanthine concentration can be oxidized via the action of xanthine oxidase to uric acid or may be salvaged back to the muscle adenine nucleotide pool (Fig. 2.1) during recovery periods. By varying the work and rest conditions of BIHIX protocols, hypoxanthine may be metabolised via different rate-dependent pathways.



1. ATPase
2. adenylate kinase
3. AMP deaminase
4. cytoplasmic 5'-nucleotidase
5. purine nucleotide phosphorylase
6. xanthine oxidase
7. 5-phosphoribosyl-1-pyrophosphate (PRPP)
8. adenylosuccinate synthetase
9. adenylosuccinate lyase
10. hypoxanthine/ guanine PRPP transferase

Figure 2.1: Major pathways of adenine nucleotide metabolism in human skeletal muscle (adapted from Stathis et al., 1994).

2.2.3 Glycolysis

Glycolysis occurs in the muscle cytosol and leads to production of two lactate (La^-) and 3 ATP molecules per glycogen unit of glucose (Sjödin, 1992; Newsholme and Leech, 1991). A cascade of 11 reaction steps is initiated by a phosphorolytic cleavage in which glucose units are split from glycogen (Lehninger, 1975). The first reaction is called glycogenolysis and is catalyzed by the enzyme glycogen phosphorylase (Sjödin, 1992; Newsholme and Leech, 1991). Glycogen phosphorylase is activated not only by hormones and inorganic ions (e.g. Ca^{2+} and Pi in the cytosol) (Chasiotis, et al., 1987), but also by a transient increase in free AMP and free ADP levels related to the ATP turnover rate (Ren and Hultman et al., 1990). The rate-regulating step in glycolysis is catalyzed by phosphofructokinase (PFK), where fructose-6-phosphate is phosphorylated by ATP to fructose-1,6-diphosphate (Newsholme and Leech, 1991). PFK has been shown to be activated by increased concentrations of metabolites, such as ADP and AMP (Katz et al., 1986a; Spriet 1987), Pi and hydrogen ions in the cytosol (Katz, 1986b). The availability of the electron carrier nicotinamide adenine nucleotide (NAD^+) may also inhibit the rate of glycolysis. NAD^+ is essential for the oxidation of glyceraldehyde-3-phosphate in glycolysis. The last step in glycolysis is the conversion of pyruvate to lactate via lactate dehydrogenase (Newsholme and Leech, 1991). Glycolysis results in an accumulation of muscle lactate which effluxes from muscle and can be detected in blood (Walsh and Bannister, 1988). The accumulation of lactate during exercise indicates that glycolysis has occurred.

Glycolysis, together with PCr, contributes to ATP production during BIHIX (Wooton et al., 1983; McCartney et al., 1986; Gaitanos et al., 1993). ATP production from

glycolysis may be estimated from changes in lactate and pyruvate by using the formula: 1.5 x Δlactate + 1.5 x Δpyruvate (Gaitanos et al. 1993). In response to a series of 10 x 6 s sprints with 30 s RI, it can be inferred that muscle lactate ($[La^-]_m$) increased steadily during the first few sprints, but did not increase further during the final sprint (Gaitanos et al., 1993). At the same time, blood lactate concentration ($[La^-]_b$) had increased to 12.6 $mmol.l^{-1}$ by the 9^{th} sprint. In BIHIX, $[La^-]_b$ is affected by the combination of work and rest, with the higher work:rest ratios generally showing higher $[La^-]_b$ (Balsom et al., 1992a & 1992b). It should be noted that for BIHIX, $[La_b^-]$ is not a good predictor of fatigue and exercise performance. This is partly due to other metabolic factors affecting performance (especially PCr) and $[La^-]_b$ not always paralleling $[La^-]_m$ (Balsom et al., 1995).

2.3 Aerobic Metabolism

Aerobic energy requires the presence of oxygen in the process of yielding energy for ATP production and uses three classes of substrates: carbohydrates, fats and protein. Aerobic metabolism requires several series of complex chemical reactions including glycolysis, the Krebs Cycle and the electron transport system (Newsholme and Leech, 1991). Both anaerobic and aerobic metabolism contribute to ATP resynthesis during exercise (Cheetham et al., 1986; McCartney et al., 1986; Fox et al., 1989; Medbö and Tabata 1989; Spriet et al., 1989). The contribution of both metabolic processes depend on the duration and intensity of exercise, but in relative terms the importance of the aerobic processes is increased with exercise duration and the associated obligatory decrease in exercise intensity (Medbö and Tabata 1989). By using the method of

calculating the relationship between the accumulated O_2 deficit and accumulated O_2 uptake and the duration, Medbö et al. (1989) quantified the relative importance of aerobic and anaerobic processes during exhaustive, supramaximal bicycle exercise. The contribution of aerobic energy release increased from 40% at 30 s duration to 50% at 1 min and to 65 % for exercise lasting 2 min (Medbö and Tabata 1989).

Although ATP turnover during BIHIX is provided mainly by PCr degradation and glycolysis (McCartney et al., 1986; Balsom et al., 1992; Gaitanos et al., 1993), some glycogen must also be used aerobically (McCartney et al., 1986; Spriet et al., 1989; Gaitanos et al., 1993; Bogdanis et al., 1996). However, very little is known about aerobic metabolism during BIHIX, partly because it is difficult to quantify. The decline in energy supply from anaerobic pathways during subsequent bouts of maximal intermittent exercise can be partly compensated from an increase in aerobic metabolism (McCartney et al., 1986; Spriet et al., 1989). Gaitanos et al. (1993) calculated that glycolysis and PCr contributed ~94% of the total ATP production in sprint 1, but fell to ~50% in sprint 10 (Table 2.2). Although this reduction in anaerobic energy yield was severe, mean power decreased to only 73% of that achieved in the first sprint. This raised the possibility that aerobic metabolism contributed significantly to the performance of the last (few) sprint(s). This was supported by Bogdanis et al. (1996), who examined the contribution of PCr and aerobic metabolism to energy supply during two 30 s sprints with a 4 min RI in between. It was reported that during the second 30 s sprint, despite ~41% reduction in ATP production calculated from the anaerobic sources, the total work done was reduced by only ~18%. This mismatch between the decrease in anaerobic energy yield and power output during the second sprint was attributed to an increased contribution of aerobic metabolism. This compensation was reflected as VO_2 was increased from 2.68 $l \cdot min^{-1}$ in

sprint 1 to 3.17 $l \cdot min^{-1}$ in sprint 2 (Bogdanis et al., 1996). During three series of BIHIX, each performed over a total distance of 600 m (ie. 40 x 15m, 20 x 30m, and 15 x 40m) and with 30s rest periods between sprints (ie work:rest ratio was varied for the three series), oxygen uptake was greater for the higher work:rest ratio (ie 15 x 40 m series) than for 40 x 15 m (Balsom et al., 1992a). A major part of the immediate post-exercise oxygen consumption has been associated with the resynthesis of PCr during recovery (Piiper et al., 1970).

2.4 Importance of Recovery Duration during Brief, Intermittent, High-Intensity Exercise

Continuous exercise is characterised simply by the two dimensions of intensity and duration, and the energy requirements of this exercise depend mainly on these two characteristics. In contrast, intermittent sports, such as hockey, soccer, football, basketball and tennis, are characterised by phases of intense exercise, punctuated by rest or lower-intensity exercise. The associated physiological responses are affected not only by exercise characteristics such as intensity and duration, but also by the recovery conditions (duration and activity). During the exercise phase of BIHIX, energy is provided primarily via anaerobic pathways (Boobis et al., 1987; Gaitanos et al., 1993), while energy for rest interval processes is derived almost exclusively from aerobic pathways (Harris et al., 1976; Colliander et al., 1988).

The influence of recovery duration on performance during repeated, short sprints has been previously studied using a cycle ergometer (Wooton et al., 1983) and on a non-motorised treadmill (Holmyard et al., 1988). These authors proposed that a reason for

the onset of fatigue was insufficient resynthesis of ATP during recovery. In a study of 15 x 40m running sprints (each sprint lasting about 6 s) with relatively long RI's of 2 min (work:rest ratio ≈ 1:20), the tenth sprint time was not significantly different from the first sprint time; that is, fatigue was only evident after the tenth sprint (Balsom et al., 1992b). In contrast, when the RI was reduced to 30 s for the same series of 15 x 40 m sprints, fatigue was evident from the third sprint. In a study of 10 x 6 s maximal sprints with 30 s RI (Gaitanos et al., 1993), the recovery intervals were sufficient to allow significant PCr resynthesis and considerable contribution from PCr to ATP production during the last of the ten sprints. From these studies, it appears that the amount of PCr resynthesis, and hence PCr availability for the 2^{nd} to n^{th} sprints, is very important for performance of BIHIX. Furthermore, 30 s RI from a 6 s sprint enables substantial, but incomplete, recovery of PCr.

Section B: Factors Affecting Muscle Creatine with Reference to Effect of Creatine Supplementation on Exercise Performance and Metabolism

2.5 Introduction

Since creatine was discovered by Chevreul in 1832 and Lieberg in 1847 (cited by Balsom et al., 1994), it has attracted interest from the scientific community due to its central role in skeletal muscle metabolism. It has been reported that over 95% of the total body creatine is located in skeletal muscle in humans, 30% of which is in its free (Cr_f) form.

The remainder of skeletal muscle creatine is present in its phosphorylated form (Balsom et al., 1994).

In humans, the normal concentration of creatine in plasma is 50 to 100 $\mu mol.l^{-1}$ and the rate of daily turnover of creatine to creatinine for a 70 kg male has been estimated to be around 1.6% per day (Harris et al., 1992; Balsom et al., 1994). Following a period of creatine supplementation (CrS), total creatine (TCr = PCr + Cr) and PCr contents were both increased in skeletal muscle (Harris et al., 1992; Greenhaff et al., 1994a; Balsom et al., 1995; Febbraio et al., 1995). No side-effects were reported with short term creatine supplementation in the doses used (Harris et al., 1992).

2.6 Age, Gender and Muscle Fibre Type

Cr and PCr levels in skeletal muscle vary in individuals and are influenced by factors such as muscle fibre type (Söderlund et al., 1992), age (Möller et al., 1980), and gender (Forsberg et al., 1991). In a group of elderly people (52 to 79 years), the level of Cr was higher than in a group of younger participants (18 to 36 years), but no differences were found in TCr level between these two groups (Möller et al., 1980). Only one study found that females usually have a higher TCr content in muscle than males (Möller et al., 1980). Tesch et al. (1989) and Söderlund et al. (1992) separated type I and type II fibres in muscle biopsy samples and demonstrated that type II fibres had a higher PCr concentration than type I fibres.

2.7 Effects of Exercise Training on Creatine Concentration in Skeletal Muscle

The average TCr content in human skeletal muscle is about 120 mmol.kg $^{-1}$ dry muscle with a range of about 70 to 150 mmol.kg $^{-1}$ dry muscle (data from 81 biopsy samples) (Harris et al., 1974). Few studies have examined the effects of exercise training on Cr and PCr content in skeletal muscle. No significant changes in skeletal muscle Cr and PCr content were reported in response to endurance training (Karlson et al., 1972) or high-intensity (sprint) training (Grimby et al., 1973; Stathis et al., 1994). In contrast, McDougall et al. (1977) reported that Cr and PCr contents in resting muscle in the upper limb increased by 39% and 22%, respectively, after 5 months of resistance training. Bernus et al. (1993) reported higher levels of PCr in the quadriceps muscles of sprinters compared with endurance runners, but this difference may have been due to a higher percentage of type II fibres in the sprinters rather than an effect of the training *per se*. In summary, it appears unlikely that endurance or sprint training increases skeletal PCr or Cr levels in individual muscle fibres.

2.8 Effects of Creatine Supplementation on Exercise Performance

2.8.1 Introduction

Following CrS, several investigators reported improved performance of high-intensity intermittent exercise (Harris et al., 1993; Greenhaff et al., 1993; Balsom et al., 1993a, 1995; Dawson et al., 1995), while others were not able to demonstrate ergogenic effects

(Green et al., 1993; Balsom et al., 1993b; Febbraio et al., 1995; Cooke et al., 1995). The lack of consensus was probably due to differences in the exercise protocols (intensity, duration, single versus intermittent exercise, work:rest ratios), the types of the experimental subjects (untrained, endurance-trained or sprint-trained) and the effectiveness of the CrS procedure in increasing TCr and PCr contents (Table 2.3). CrS does appear to improve work and power output for BIHIX, but not for endurance exercise and its effects are equivocal for single sprint performance. CrS has recently received much interest from the sports and science communities, because it is safe, with no documented side-effects in the recommended doses, except for mild fluid retention, and is not presently on the list of IOC banned substances.

Author(s)	Subject		CrS		Exercise Protocol	Performance		Physiological	
	Sex	Training status	Dose / day	Days		Measure	Results (After CrS)	Measure	Results (After CrS)
Harris et al., 1992	5 F & 12 M	varied	4 x 5g	4,5,7 & 21	5 subjects performed one leg cycling for 1h/day during CrS			muscle TCr, ATP & PCr	~ 20% ↑ TCr & > 20% of ↑ PCr ↑ Cr more if performed with ex
Harris et al., 1993	10 F	trained middle distance	6 x 5 g	6	2 series @: 4 x 300 m & @: 4 x 1000 m with 4 & 3 min RI, respectively.	run time	↓ run times for both distances.		
Balsom et al., 1993 a	16 M	active	5 x 6g	6	2 series of 10 x 6 s sprint with 30 s RI: EX_{130} (130 rev. min^{-1}); EX_{140} (140 rev. min^{-1})	WO and PPO for EX_{140}	EX_{130} : n/a EX_{140} ↑ WO and PPO	blood La^-, plasma hypox & VO_2	EX_{130}: ↓ VO_2, ↓ La_b^-, ↓ hypox EX_{140}: ↓ hypox & La_b^-, no Δ in VO_2
Balsom et al., 1995	7 M	highly physically active	4 x 5g	6	5 x 6 s sprint with 30 s RI followed (40 s later) by 1 x 10 s sprint at 140 rev. min^{-1}	PPO	better maintenance of PPO	TCr, PCr, ATP La_b^-, plasma hypox	~18% ↑ in resting TCr; after 5th sprint↑ PCr & ↓ La_m^-; no Δ in La_b^- & plasma hypox
Dawson et al., 1995	40 M	active	4 x 5g	5	Study 1 (S_1): 10 s max. sprint Study 2 (S_2): 6 x 6 s max. sprint	WO & PPO	S_1: No Δ with CrS S_2: ↑ PPO, WO for CrS	La_b^- & pH pre ex. & at 1, 3, 5, & 7 min post ex.	No Δ in post-ex La_b^- & pH
Greenhaff et al., 1994 a	8 M	active	4 x 5g	5	20 x 1.6 s with 1.6 s rest of intense electrically-evoked isometric contractions			ATP, PCr, TCr & La_m^- at 0, 20, 60, 120 s of recovery	~20%↑ in TCr at rest; ↑ PCr resynthesis at 2 min and ↑ La_m^- at 0 s of recovery

Table 2.3: Summary of the literature on the effects of CrS on exercise performance and metabolism.
Female (F), male (M), exercise (ex), peak power (PPO), mean power (MPO), work (WO), fatigue index (FI), total creatine (TCr), phosphocreatine (PCr), blood lactate (La_b^-), muscle lactate (La_m^-), hypoxanthine (hypox) & ammonia (NH_3^+)

Greenhaff et al., 1994 b	6 M	active	4 x 5g	5	2 x 30 s maximal cycling with 4 min RI.	WO	↑ WO	ATP, PCr, TCr & La_m^-	ATP degradation reduced 50% in the second bout. No Δ in La_m^-
Greenhaff et al., 1993	3 F & 9 M	active	4 x 6g	5	5 x 30 maximal voluntary contractions with 1 min RI	peak torque	↑ peak torque	La_b^-, plasma NH_3^+	↓ plasma NH_3^+ no Δ in La_b^-
Birch et al., 1994	14 M	active	4 x 5g	5	3 x 30 s max. ex with 4 RI	WO, PPO & MPO	↑ WO, PPO & MPO in bouts 1 & 2 only	plasma NH_3^+ & La_b^-	No Δ in La_b^-, ↓ plasma NH_3^+
Cooke et al., 1995	12 M	untrained	4 x 5g	5	2 x 15 s with 20 min RI	PPO, WO & FI	No Δ in PPO, WO & FI		
Febbraio et al., 1995	6 M	untrained	4 x 5g	5	4 x 1 min at about 120% of VO_{2max} with 1 min RI followed by a fifth bout to fatigue	time to fatigue in 5th bout	CrS had no effect on performance	TCr, PCr, ATP, ADP, pH, La_m^-, plasma NH_3^+ & La_b^-	↑ TCr & PCr; no Δ in La_b^- & plasma NH_3^+ After 28 days, TCr returned to basal levels
Green et al., 1993	8 M	active	4 x 5g	5	A treadmill run at 80% of VO_2, followed 5 bouts of isometric contraction at 70% with 3 min RI	total isometric endurance time	CrS had no effect on performance	VO_2, & La_b^-	No Δ
Balsom et al., 1993 b	18 M	active; some were well-trained	4 x 5g	6	A treadmill run to exhaustion at ~120% of VO_{2max} & ~ 6 km run	run time	No Δ	VO_2, La_b^- & plasma hypox	No Δ except ↑ La_b^- with the treadmill run after CrS

Table 2.3: Continued

2.8.2 Effect of CrS on a Single Bout of High-Intensity Exercise

Most studies of the effects of CrS were conducted using BIHIX protocols (see 2.8.3, below), rather than a single bout of intense exercise. Studies that used single bouts of exercise have been equivocal in terms of the ergogenic effects of CrS, with Cooke et al. (1995) not showing increased power or decreased fatigue index for a 15 s cycle sprint in untrained men. Dawson et al. (1995) reported that total work and peak power were improved during a 10 s cycle sprint after creatine supplementation, but a similar response occurred with their placebo treatment. Interestingly, the performance of the first repetition of 6 x 6 s sprints was improved following CrS, but not with placebo. A mechanism that has been proposed to explain increased power after CrS for a single sprint, or the first of a series, is that CrS increases TCr and PCr contents in muscle leading to higher initial PCr availability and higher ATP turnover during sprinting (Greenhaff, 1995).

2.8.3 Effect of CrS on Brief, Intermittent, High-Intensity Exercise

a) Repeated Short Sprints (≤ 30 s)

Several groups have recently reported that CrS improves performance of BIHIX where the duration of each effort is less than 30 s (see Table 2.3, above). Following CrS (4-5 g/day for 5 days), muscle torque production was higher after CrS towards the end of each of five bouts of 30 maximal voluntary contractions, with 60 s RI (Greenhaff et al., 1993). CrS also enhanced performance of 10 x 6 s high-intensity exercise with 30s RI (Balsom et al., 1993a). Using a similar exercise protocol of 6 x 6 s cycle sprints,

departing every 30 s, Dawson et al. (1995) found that work and peak power were higher after CrS.

b) Sustained Speed (≥ 30 s to 60 s repeats)

There have been few studies which investigated this ergogenic effect on performance of BIHIX with longer work and rest duration. Febbraio et al. (1995; Table 2.3) investigated the effect of CrS on the performance of 4 x 1 min cycling bouts at about 120% of VO_{2max} with 1 min RI, followed by a fifth bout at the same intensity to fatigue. CrS increased TCr and PCr contents (Febbraio, personal communication) but did not increase the time to fatigue during the fifth bout. Harris et al. (1993) investigated the effects of CrS on the performance of 4 x 300 m runs with 4 min RI and 4 x 1000 runs with 3 min RI. The run times were significantly decreased (improved) following CrS, but only by 0.3 s (0.8%) and 2.1 s (1.1%), respectively. This suggests that CrS is effective for exercise of this intensity and duration, but the effect sizes were relatively small.

2.8.4 Effect of CrS on Prolonged Continuous Exercise

During prolonged continuous exercise, ATP is produced primarily via oxidative phosphorylation, with smaller contributions from anaerobic sources when exercise intensity fluctuates, as in terrain running (Söderlund et al., 1991; Balsom et al., 1993b). Balsom et al (1993b) investigated the influence of CrS on performance during a run at 120% of $\dot{V}O_{2max}$ on a motor-driven treadmill until exhaustion and a 6 km terrain run. There was no improvement in performance in either of these tests following CrS. In fact, run time was significantly greater for the 6 km run after Cr feeding. This was in agreement with the study of Green et al. (1993) who examined the influence of CrS on

metabolism during sub-maximal isometric endurance exercise (Table 2.3). The total endurance time (sum of all five bouts) was significantly increased after Cr ingestion. It was suggested that the decreased performance could have been due to the increase in body mass typically seen with CrS (Balsom et al., 1993b). $\dot{V}O_{2max}$ also did not change following the administration period (Balsom et al., 1993b). The ergogenic effects of CrS on exercise performance and metabolism appear to be restricted mainly to BIHIX. However, the exact mechanisms by which CrS affects performance and metabolism and the kind(s) of exercise for which CrS is more effective are unclear, and await further investigation.

2.9 Effects of CrS on Exercise Metabolism

2.9.1 Overview

Several inter-related metabolic mechanisms have been proposed to explain the ergogenic effects of CrS during BIHIX. Firstly, CrS results in an increase in total creatine concentration of some 20%, with a smaller increase in PCr (Harris et al., 1992; Febbraio et al., 1995, Hultman et al., 1996). PCr availability is important for ATP resynthesis (Hultman et al., 1967) and resistance to fatigue (Hultman et al., 1990) during intermittent, high-intensity exercise. Increased PCr concentration at the commencement of HIX increases the capacity of the creatine kinase reaction to resynthesise ATP (Hultman et al., 1967). In a study of intense electrically-evoked stimulation of skeletal muscle, PCr utilisation was apparently greater following CrS (Greenhaff et al., 1994b), assuming that pre-exercise PCr content after CrS was higher in their subjects (pre-

exercise PCr content was not measured). PCr is not only an immediate buffer for ATP resynthesis, but is also thought to facilitate energy translocation from mitochondria to sites of ATP utilization (Davies, 1965; Kammermeter, 1987; Bessman, 1985). Thus it has been hypothesised that by increasing PCr content, this energy transfer may be enhanced (Balsom, et al., 1994).

Secondly, PCr resynthesis has been reported to increase during the recovery intervals in intermittent, high-intensity exercise following CrS (Greenhaff et al., 1994a), although the rate of PCr resynthesis did not increase above the control rate until the second minute of recovery. It is also likely that PCr resynthesis increased during the recovery intervals in a series of 5 x 6 s sprints with 30 s RI (Balsom et al., 1995), although due to the timing of the muscle biopsy samples, it is not possible to rule out the higher initial PCr content to explain the higher PCr content at the end of these sprints. The advantage of increasing PCr resynthesis during BIHIX is that it has the effect of increasing PCr content at the start of the 2^{nd} to n^{th} sprints, thereby maintaining ATP resynthesis during these sprints. Thirdly, it has been estimated that the typical increase in PCr content following CrS would, in turn, increase the muscle buffering capacity by approximately 7% (Greenhaff et al., 1994a, based on measurements by Sahlin and Hultman, 1980). Fourthly, it is thought that the adenine nucleotide pool is conserved better during high-intensity exercise with CrS: the higher PCr content at the start of each sprint helps to maintain ATP resynthesis through the creatine kinase reaction and so there is less reliance on the adenylate kinase reaction. This was likely to be the explanation for lower circulating concentrations of ammonia (Greenhaff et al., 1993) and hypoxanthine (Balsom et al., 1993a) after CrS, even though work and power were higher in these studies. Fifthly, the higher capacity of the creatine kinase system following CrS reduces the need for glycolysis during high-intensity exercise, as evidenced by lower concentrations of lactate in muscle (Balsom et

al., 1995) and plasma (Balsom et al., 1993a). Sixthly, CrS administered to people suffering from gyrate atrophy caused increases in the diameters of Type II fibres (Sipila et al., 1981), however, the hypertrophy was in response to long term feeding and so is unlikely to apply for the short term creatine feeding program that has been used in most studies (note: although it is generally recognised that the usual CrS protocol causes a gain in body mass of about 1 kg, this is almost certainly due to water retention, rather than acute increases in muscle lean mass; Hultman et al., 1996).

2.9.2 Effects of CrS on Total Creatine and PhosphoCreatine Contents in Muscle

The average TCr concentration in human skeletal muscle without CrS is about 120 mmol·kg^{-1} dry muscle, with an upper limit of the order of 150 mmol·kg^{-1}. dry muscle (Harris et al., 1974). A similar upper limit has been suggested following CrS, and people with the lowest initial levels of muscle creatine concentration, tend to exhibit the greatest increases in muscle TCr following CrS (Harris et al., 1992).

In the isotope ^{15}N-labelled Cr dilution study of Crim et al. (1976), creatinine excretion was measured in subjects loaded with 10g of creatine per day. They reported increases in renal excretion of creatinine and concluded that the TCr in the body may be increased by dietary intake. A recent study which reported the effects of CrS on muscle Cr content (Harris et al., 1992), examined 17 healthy men and women aged 20-62 years, with variable levels of fitness. TCr content in resting muscle increased by some 20-40%, with PCr content increasing by approximately 20% after CrS. The mean TCr content was increased from 126.8 to 148.6 mmol·kg^{-1} after CrS. The increase in the TCr pool resulted from increases in both PCr (84.2 to 90.6 mmol·kg^{-1}) and Cr (42.6 to 58.0

mmol·kg^{-1}) contents. The increase in muscle TCr content was greater in a second study, when the subjects exercised only one leg for one hour per day during the period of CrS. The mean TCr content was increased from 118.1 mmol·kg^{-1} pre CrS to 148.5 mmol·kg^{-1} in the rested leg and to 162.2 mmol·kg^{-1} in the exercised leg. The increases in TCr appeared more dependent upon the initial TCr content than on daily dose rate and duration of CrS. The highest PCr final content was recorded in a vegetarian with the lowest initial Cr levels. The study demonstrated that 20 to 40 % of the increase in TCr content was in the form of PCr with the ingestion of 4 to 6 x 5g doses of Cr monohydrate per day for more than 2 days. However, no statistical analyses were performed by Harris et al. (1992). In addition, the subjects of the study were divided into subgroups to undergo different protocols of Cr administration, thereby, reducing the statistical power of the study. Despite these limitations, most researchers of the effects of CrS on exercise performance and metabolism have used procedures based on the study by Harris et al. (1992).

The increases in TCr and PCr contents in muscle following CrS reported by Harris et al. (1992) are supported by several other groups (Greenhaff et al., 1994a; Balsom et al., 1995; Febbraio et al., 1995; Hultman et al., 1996), but not all subjects demonstrated the same increases in TCr or PCr in response to CrS (Greenhaff et al., 1994a). Subjects with the lowest initial TCr content (<120 mmol·kg^{-1} dry muscle) experienced the greatest increases in TCr (25-35 mmol·kg^{-1} dry muscle), equivalent to 20-35% of their initial TCr content, while three subjects were classified as non-responders (Greenhaff et al., 1994a). Following CrS, TCr content in muscle gradually declined to pre-supplementation levels after about 28 days (Febbraio et al., 1995; Hultman et al., 1996). It is important to point out that the increases in PCr content after CrS reported in the literature (average of

10%) are not as large as the increases in TCr (average rise of 20%). Some studies have reported a significant increase in resting PCr content in muscle after CrS (Harris et al., 1992; Febbraio et al., 1995; Hultman et al., 1996), whilst others found no significant change in resting muscle PCr content (Greenhaff et al., 1994a; Balsom et al., 1995; Snow et al., 1996). A possible explanation is that most of the increases in TCr content after CrS were due to increases in Cr content, with smaller increases in PCr which have proved difficult to detect.

It has been reported that an upper limit of Cr storage in human muscle is about 150-160 $mmol \cdot kg^{-1}$ dM (Harris et al., 1992; Greenhaff et al., 1994a), and this seems to apply with and without Cr supplementation. This explains why some people fail to demonstrate increases in TCr, while others respond markedly to CrS. There have been no reports of CrS causing increases in ATP content in muscle (Harris et al., 1992; Balsom et al., 1994; Maughan 1995; Febbraio et al., 1995).

2.9.3 Effects of CrS on PCr Resynthesis

One of the most likely explanations for an ergogenic effect of Cr ingestion during BIHIX is that PCr resynthesis is accelerated during rest intervals between exercise bouts (Balsom et al., 1995; Greenhaff et al., 1993). This would lead to better maintenance of ATP production during subsequent exercise via the creatine kinase system. In order to investigate the effect of oral Cr ingestion on PCr resynthesis, Greenhaff et al. (1994a) calculated the rate of PCr resynthesis during recovery from intense isometric contractions. In five of eight subjects, CrS ($20g \cdot day^{-1}$ for 5 days) substantially increased muscle TCr and accelerated PCr resynthesis from 60 to 120 s of recovery. In a study of the effects of CrS on 5 x 6 s sprints with 30 s RI, PCr content had fallen immediately

after the last sprint by 34 mmol·kg^{-1} without CrS, but by only 19 mmol.kg^{-1} with CrS (Balsom et al., 1995). Due to the timing of the muscle biopsies in this study, it was not possible to estimate the contributions of PCr resynthesis and utilisation to the overall increase in PCr availability.

2.9.4 Effects of CrS on Adenine Nucleotide Metabolism

a) Muscle ATP

It appears that muscle ATP content in resting muscle does not increase after CrS (Harris et al., 1992; Balsom et al., 1995). However, muscle ATP degradation during intermittent, intense exercise does appear to be reduced with CrS (Greenhaff et al., 1994b). They reported that Cr ingestion resulted in a 50% reduction in muscle ATP loss during a second bout of maximal exercise, with work output being increased by about 6%. It was suggested that CrS maintained ATP turnover during intense muscle contraction, due to better maintenance of ATP resynthesis from ADP.

b) Plasma Ammonia

Increases in plasma ammonia concentration during maximal exercise have been attributed to loss of adenine nucleotides from skeletal muscle (Lowenstein et al., 1972; Greenhaff et al., 1993), as anaerobic metabolism shifts from the creatine kinase to the adenylate kinase reaction (Balsom et al., 1992a). The influence of CrS on plasma ammonia accumulation during repeated bouts of 30 maximal voluntary contractions was investigated by Greenhaff et al. (1993). Plasma ammonia accumulation was lower during exercise and recovery when the subjects were supplemented with creatine, which the authors hypothesised as being due to an enhanced rate of ATP resynthesis and lower rate

of AMP deamination during exercise. This suggests that ADP rephosphorylation to ATP was greater after CrS due to greater initial availability of PCr during exercise. Birch et al. (1993) also found lower plasma ammonia concentrations during repeated bouts of maximal isokinetic cycling after Cr ingestion. In a study of the effects of CrS on 10 x 6s of high-intensity cycling with 30 s RI, plasma hypoxanthine accumulation was lower following CrS, even though more work was performed, reflecting a lower rate of adenine nucleotide degradation (Balsom et al. (1993a).

2.9.5 Effects of CrS on Glycolysis

During BIHIX without CrS, blood lactate concentration ($[La^-]_b$) has been demonstrated to increase during and immediately following exercise, compared with resting $[La^-]_b$ (Wootton et al., 1983; McCartney et al., 1986; Balsom et al., 1992a & b; Gaitanos et al., 1993). After Cr loading, $[La^-]_b$ was unchanged from pre-loading values during maximal intermittent exercise, in spite of more work being accomplished (Greenhaff et al., 1993; Birch et al., 1993; Dawson et al., 1995). These findings suggested that the increases in work output were not due to increased contributions from glycolysis. Using a different approach of repeated 6s cycle sprints with 30 s RI at a constant high intensity, Balsom et al. (1993a) reported lower $[La^-]_b$ after CrS. Taken together, these studies suggest that glycolysis is lower at given absolute workloads after CrS, and no higher at maximal exercise, in spite of the higher work being done. However, a problem with this interpretation is that all of these studies used blood analyses to infer what was happening in muscle.

There have been few studies reporting the effects of CrS on muscle lactate concentration ($[La^-]_m$) during BIHIX. Greenhaff et al. (1994a) found after CrS, $[La^-]_m$ was higher

immediately after a given intensity of electrically-evoked isometric contractions than for placebo. This did not concur with their other published findings in response to maximal voluntary contractions in which they reported no changes in $[La\text{-}]_b$ (Greenhaff et al., 1993) or $[La^-]_m$ (Greenhaff et al., 1994b), when the work performed was higher following CrS in each case. They attributed the inconsistent findings in the electrically-stimulated muscle to a technical problem with force measurement and claimed that the force produced with and without CrS may not have been the same, as was originally intended (Greenhaff et al., 1994a). Balsom et al. (1995) found that $[La^-]_m$ was significantly lower after CrS for 5 x 6 s sprints with 30 s RI at a constant intensity. They also found that for a maximal sprint following the first five sprints, $[La^-]_m$ tended to be lower, even though power was increased with CrS. These results suggested that muscle lactate production was lower after CrS for high intensity exercise. However, even these conclusions need to be qualified, since lactate efflux from muscle was not measured in any of these studies. If efflux was higher after CrS, due to higher lactate production, then $[La^-]_m$ could be lower or unchanged, thereby masking the higher glycolysis.

2.9.6 Effects of CrS on Aerobic Metabolism

ATP resynthesis during high-intensity exercise depends more on anaerobic than aerobic mechanisms for brief, high-intensity exercise, with a shift towards oxidative mechanisms as the duration of exercise increases (McCartney et al., 1986; Spriet et al., 1989). It was demonstrated that aerobic metabolism contributed to energy provision during the latter stages of BIHIX (Piiper et al., 1970; Gaitanos et al., 1993; Bogdanis et al., 1996). However, these studies used pulmonary (whole body) $\dot{V}O_2$ and so it is not certain whether muscle oxygen consumption also increased, although that is likely. Only one

study investigated the effects of CrS on aerobic metabolism during BIHIX (Balsom et al., 1993a; Table 2.3). They measured pulmonary VO_2 at the start of exercise and at the end of the recovery for the 7^{th}, 8^{th} and 9^{th} sprints before and after supplementation. VO_2 was significantly lower after CrS for the same exercise intensity. This finding was surprising, given that PCr resynthesis is dependent upon oxygen delivery to muscle (Sahlin et al., 1979), and it is likely that PCr resynthesis increased after CrS (Greenhaff et al., 1994a; Balsom et al., 1995). Thus, oxygen uptake would be expected to increase. However, the results of this study may have been influenced by the measurement of pulmonary rather than muscle VO_2. Therefore, the effects of CrS on aerobic metabolism during BIHIX remain unclear.

Section C: Aims and Hypotheses

2.10 Aims

2.10.1 General Aims:

To determine whether CrS enhances the capacity to perform brief, intermittent, high-intensity exercise with widely different rest intervals.

2.10.2 Specific aim:

To compare the metabolic and ergogenic benefits of CrS during brief, intermittent, high-intensity exercise with rest intervals of either 30 seconds or 6 minutes, using the same set of repeated cycle sprints (10 x 6 second sprints).

2.11 Hypotheses

1. That CrS will enhance the performance of 10 x 6 s sprints with rest intervals of 30 s.

2. That CrS will enhance the performance of 10 x 6 s sprints with rest intervals of 6 min.

3. That the ergogenic effect will be greater for the 30 s rest interval.

4. That plasma ammonia concentration will be reduced after CrS for both rest intervals.

5. That plasma lactate concentration will not be significantly changed after CrS for both rest intervals.

CHAPTER 3 METHODOLOGY

3.1 Subjects

Fourteen physically-active male volunteers were recruited after they were informed of the nature of the investigation and possible risks involved in the procedures and they had signed a letter of consent (Appendix A). All experimental protocols were approved by the Human Research Ethics Committee of Victoria University of Technology. Physical characteristics of the subjects (age, body mass, height, $\dot{V}O_{2peak}$; mean $\pm$ SEM) were not significantly different for the creatine supplementation group (CrS) and control group (CON) (Table 3.1).

Table 3.1 Physical characteristics of the subjects. Group data is summarised as mean (SEM).

GROUP	n	AGE (years)	BODY MASS (kg)	HEIGHT (cm)	$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)
CrS	7	20.9 (0.6)	73.7 (2.4)	174.1 (1.4)	59.9 (2.4)
CON	7	21.5 (1.2)	73.6 (3.5)	177.8 (2.4)	58.3 (3.2)

3.2 Experimental Design

Subjects initially underwent an incremental exercise test to determine $\dot{V}O_{2peak}$, followed one week later by the first of two sprint series consisting of 10 x 6 s maximal intensity cycling exercise. The series of sprints were interrupted by 30 s rest intervals (RI) on one

occasion and 6 min RI on another occasion (48 hours apart, random order; Table 3.2). The 14 subjects were then divided into two groups (CrS and CON); groups were matched on the basis of the summation of the work accomplished during the baseline sprint series. The researchers and subjects were blinded as to the allocations to creatine (CrS) or placebo (CON) treatments. After one week's rest, the subjects underwent five days of the respective treatment. They then repeated the two sprint series in the same order as before supplementation, again with an intervening rest period of two days (Table 3.2). Each subject refrained from exercise and alcohol in the 24h-period prior to each trial, but apart from these restrictions, they continued their normal daily activities. Diet was not restricted or prescribed before exercise, except that subjects were requested to follow a similar eating and drinking pattern on the day of a test. For each subject, the four exercise tests were conducted at the same hour of the day.

TABLE 3.2 ***Research Design***

Week	**1**	**2**	**2**	**3**	**4**	**5**	**5**
Day	**Monday**	**Monday**	**Wednesday**		**Wednesday to Sunday**	**Monday**	**Wednesday**
Test type	$\dot{V}O_{2peak}$	**10 x 6 s sprints with either 30 s or 6 min RI**	**10 x 6 s sprints with either 30 s or 6 min RI**	**NONE**	**Creatine or placebo supplement**	**10 x 6 s sprints with either 30 s or 6 min RI**	**10 x 6 s sprints with either 30 s or 6 min RI**

3.3 $\dot{V}O_{2peak}$ Test

All subjects completed an incremental exercise test on a mechanically-braked cycle ergometer (Monark Ergogenics 868, Varberg, Sweden) to determine $\dot{V}O_{2peak}$ before allocation to either the CrS or CON groups. $\dot{V}O_{2peak}$ was not significantly different between the two groups (Table 3.1); the method is described in Appendix A.

3.4 Creatine or Placebo Supplementation Procedure

The Cr supplementation procedure was based on the work of Harris et al. (1992). After performing the first two series of sprints with 30 s and 6 min RI's, the subjects were administered 5 doses·day^{-1} for 5 days of either 5g of Cr monohydrate plus 1g dextrose (creatine group, n = 7) or 6g of dextrose (placebo group, n = 7), with each dose consumed 3 to 4 hours apart. These compounds were dissolved in 300 ml warm fluid to prevent formation of creatinine from Cr (Harris et al., 1992). Subjects were contacted by telephone to check that they were conforming to the supplementation procedures.

3.5 Sprint Tests

All subjects underwent familiarisation with the procedure of repeated 6 s sprints at the time of the $\dot{V}O_{2peak}$ tests. An air-braked front-loading cycle ergometer (Series A, Repco, Melbourne, Australia) was used for all sprint tests. A calibration rig was not available for the study. The same ergometer was used throughout the study, and no other exercise tests were performed on it during this time. However, changes in mechanical efficiency can not be ruled out. Each sprint was conducted with the subject in a standing position,

including the start, with feet secured to the pedals by toe clips and heel straps. Electrodes were attached to the chest (limb lead configuration) and connected to an electrocardiograph for heart rate and rhythm monitoring (X-SCRIBE™; Mortara, Milwaukee, USA). A catheter was then inserted into a dorsal hand vein and 10 minutes later, a resting blood sample was obtained. Subjects then performed a warm-up consisting of 2 min of gentle cycling at 50 W before a maximal intensity 3 s cycle sprint; they then cycled for a further 2 min at 50 W, before sitting quietly for 3 min. During the main series of sprints, subjects were instructed to stand on the pedals 10 s prior to each sprint and to commence the sprint with the same leg forward. At the end of each sprint, they were requested to sit passively. They were verbally encouraged to give maximal effort in all sprints during each test. One person was responsible for giving this verbal encouragement to subjects throughout the study. Blood samples were taken immediately after the fifth and tenth sprints and for 20 min after the last sprint (see Section 3.6 and Fig. 3.1).

Power output was measured using a cubic function of the flywheel velocity which was determined using a tachometer (hall effect device and a cog at the wheel hub). Peak power (PPO) and mean power (MPO) for each sprint were calculated by a microcomputer during each trial. PPO and MPO were determined as the highest and average power, respectively, during each of the 10 x 6 s sprints.

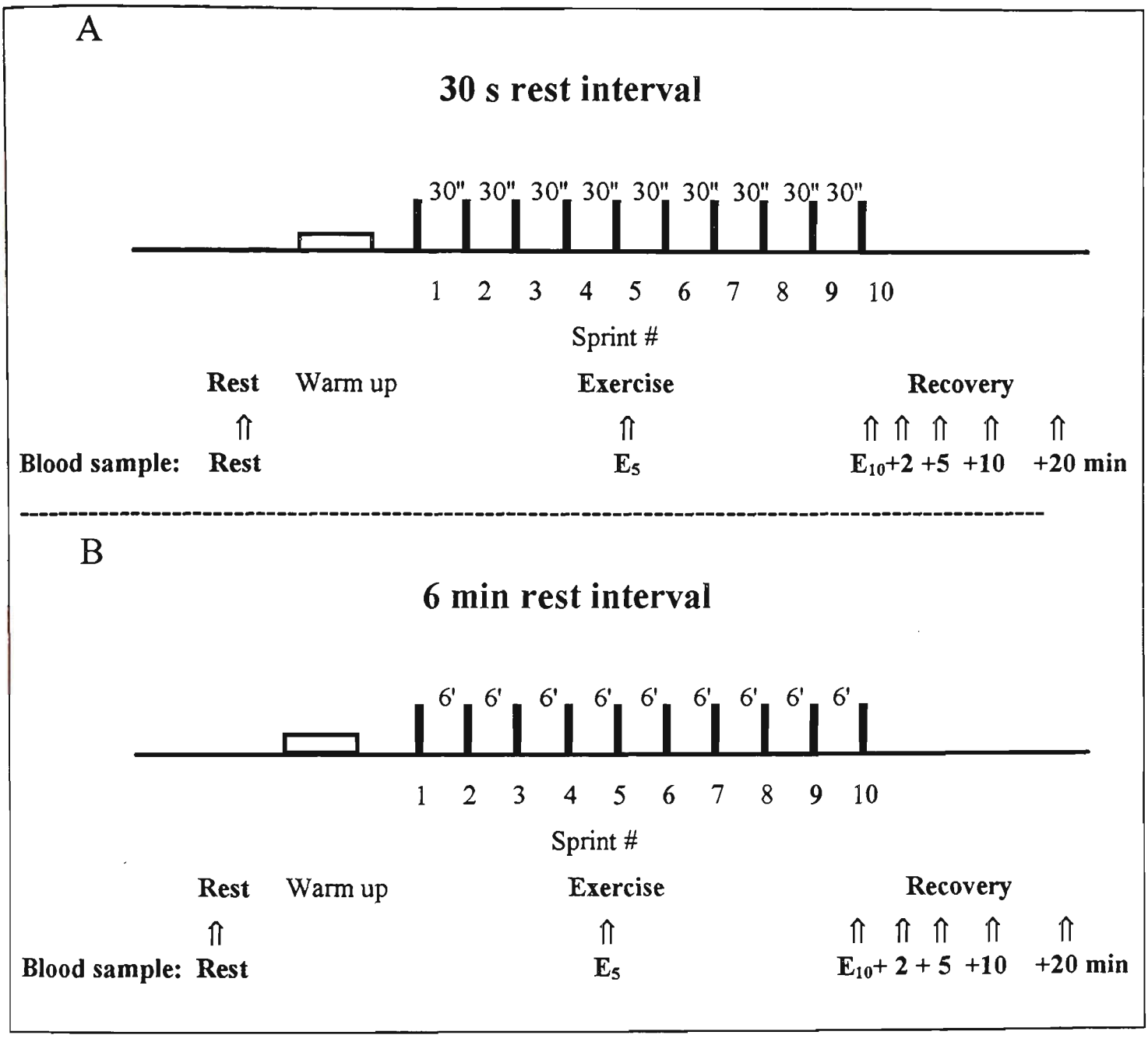


Figure 3.1: Experimental design within each series of sprints, including the timing of blood sampling for (A) 30 s RI and (B) 6 min RI.

▭ = warm up; 1, 2, 3...10 = sprint #

30" = **30 s** rest intervals between sprints

6' = **6 min** rest intervals between sprints

E_5 = sprint number 5

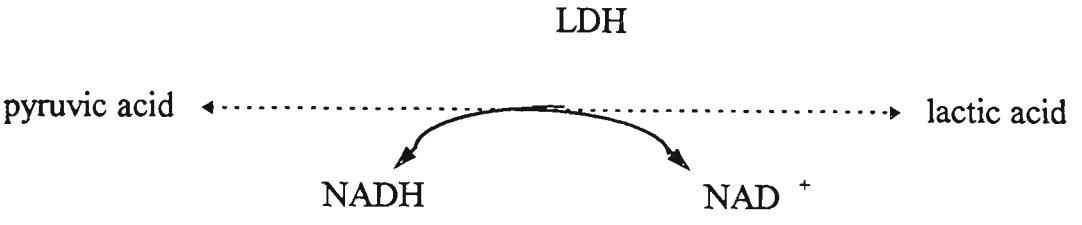
E_{10} = sprint number 10

3.6 Blood sampling, processing and analyses

Venous catheterisation: one hand and forearm were pre-warmed and then a 22-gauge teflon catheter (Jelco, Johnson & Johnson, USA) was introduced aseptically into a dorsal hand vein. A minimum volume extension tube (Tuta, 0.3 ml deadspace) with a three-way stopcock was connected to the catheter for ease of sampling. The catheter entry point was covered by "second skin" (Tegaderm, 3M, Ontario, Canada) and the hand was placed in a loose-fitting rubber glove. Patency was maintained between samples by filling the catheter and extension tube with heparinised saline (0.5 ml, 10 $IU \cdot ml^{-1}$ heparin). To arterialise the venous blood, the hand was placed in a warm water bath (45 ^{0}C) during each rest interval, according to the method of McLoughlin et al. (1992), but not during the 6 s sprints. Hand warming was also maintained for 10 minutes prior to the first sprint and for 20 minutes after the last.

Arterialised venous blood samples were taken at rest, immediately after sprints 5 and 10, and at 2, 5, 10, and 20 min of recovery (Figure 3.1). The first part of the sample (1.5 ml) was drawn anaerobically into a 3 ml heparinised syringe (Rapidlyte, 7 IU heparin), mixed and placed in an ice/water sludge until analysis in duplicate for plasma $[H^{+}]$, using an automated blood gas electrolyte analyser (Ciba Corning 865, Diagnostics Group, Medfield, MA, USA). The second part of the sample (8 ml) was drawn into a plain syringe, transferred immediately to a lithium heparin tube (10 IU heparin), mixed and placed in the ice/water sludge, then transferred to 1.5 ml Eppendorf tubes and centrifuged (15,000 G) for 3 min. An aliquot of this plasma (200 µl) was transferred to 600 µl of cold 3M perchloric acid (PCA), mixed vigorously and centrifuged (15,000 G) for 2 min. The resultant supernatant was frozen at -80 °C until analysis in duplicate for

plasma lactate concentration, based on the following reaction (Lowry and Passonneau 1972):



The remaining plasma was frozen in liquid nitrogen, stored at -80 °C and analysed in duplicate within 72 hours for plasma ammonia concentration. The method was based on reductive amination of 2-oxoglutarate, using glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH), according to the following reaction:

$$\text{2-oxoglutarate} + NH_3 + \text{NADPH} \xrightarrow{\text{GLDH}} \text{Glutamate} + \text{NADP}$$

The decrease in absorbance at 340 nm (A_{340}), due to oxidation of NADPH, is proportional to the plasma ammonia concentration. The same spectrophotometer was used for lactate and ammonia assays (Cobas Bio, Roche, Nutley, N.J., USA). Standard curves for the ammonia and lactate assays are given in Appendix B.

3.7 Statistical Analyses

PPO and MPO were analysed to test for significant differences between the CrS and CON groups (after subject allocations) prior to supplementation: two-way (GROUP and RI) analyses of variance (ANOVA), with repeated measures (SPRINT) showed that there were no significant differences between CrS and CON for either PPO or MPO

prior to supplementation. The two groups were then analysed independently for the effects of supplementation. For PPO and MPO, two-way (PRE-POST, RI) ANOVA with repeated measures (SPRINT) were applied. Similarly for the blood analyses, two-way (PRE-POST, RI) ANOVA with repeated measures (SAMPLE) were performed.

In the case of significant main effects, post-hoc (Tukey) analysis was used to locate the differences. The level of significance used to reject the null hypothesis was set at $p < 0.05$. All values are reported as means ± standard error of the mean (SEM).

CHAPTER 4 RESULTS

4.1 Sprint Performance

4.1.1 Peak Power

Pre-supplementation: There was no significant difference between the CrS and CON groups for PPO for either 30 s or 6 min RI. PPO did not decline significantly during the series of 10 sprints with the 6 min RI for either group (Fig. 4.1. A & C). In contrast, PPO declined progressively during the first eight sprints for the 30 s RI ($P < 0.01$), with no further decline thereafter (Fig. 4.1. B & D). For the series of 10 sprints, there was a significant main effect for RI within each group, with PPO being significantly higher for the 6 min than for the 30s RI ($P < 0.01$; Fig. 4.1).

Post-supplementation: In the CON group, there were no significant changes in PPO for either the 30 s or 6 min RI following placebo supplementation (Fig 4.1 C & D). In the CrS group, there were significant main effects (post- versus pre-supplementation) for both RI. For 6 min RI, PPO increased from 903 ± 32 (pre) to 964 ± 51 (post) (mean ± SEM for all 10 sprints combined; $P < 0.05$; Fig. 4.1 A). Similarly for 30 s RI, PPO increased from 826 ± 28 to 897 ± 41 ($P < 0.05$; Fig. 4.1 B). However, the decline in PPO during the 10 sprints for 30 s RI was not affected by supplementation in either group. There was no significant interaction between RI and PRE-POST (pre- versus post-treatment) for either CrS or CON group.

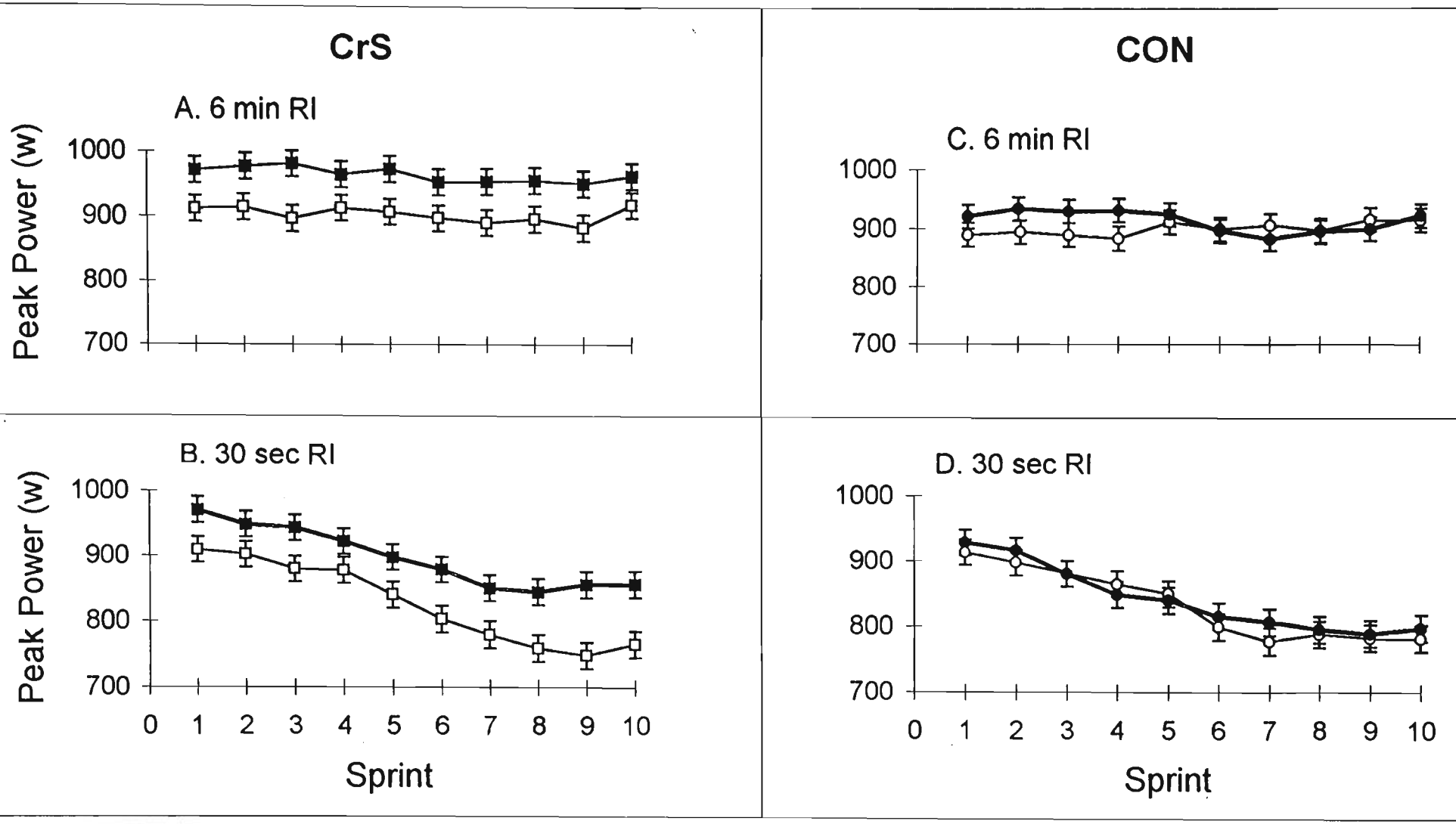


Figure 4.1 Peak Power Output (PPO) during 10 x 6 s maximal sprints, comparing post vs pre supplementation within each group for both 30 s and 6 min rest interval (RI). A & B; creatine group (CrS) (n = 7; mean and SEM): post (–■–) vs pre (–□–). PPO was significantly greater after creatine (Cr) supplementation ($P < 0.05$). C & D; control group (CON) (n = 7; mean and SEM): post (–●–) vs pre (–○–). PPO did not differ significantly after placebo (PL) supplementation.

4.1.2 Mean Power

Pre-supplementation: there were no significant differences in MPO between the CrS and CON groups for either 30 s or 6 min RI. For both groups, MPO for the 30 s RI was maintained for the first five sprints, then fell during the next three ($P < 0.01$), before being maintained for the last two (Fig 4.2. B & D). In contrast, for the 6 min RI, MPO did not change significantly over the course of 10 sprints (Fig 4.2. A & C). For the series of 10 sprints, there was a significant main effect for RI: within each group, MPO was significantly higher during the 6 min than the 30s RI ($P < 0.01$; Fig. 4.2).

Post-supplementation: In the CON group, there were no significant increases of MPO for either the 30 s or 6 min RI (Fig 4.2. C & D). In the CrS group, MPO tended to increase for both RI: in the 6 min RI, MPO increased from 646 ± 22 (pre) to 690 ± 35 (post) (mean ± SEM for all 10 sprints combined; $P = 0.054$; Fig. 4.2 A). Similarly in the 30 s RI, MPO increased from 585 ± 16 to 631 ± 28 ($P = 0.059$, Fig. 4.2. B).

4.2 Plasma Metabolites

4.2.1 Ammonia

Pre-supplementation: There was no significant difference between the CrS and CON groups for plasma ammonia concentration ($[NH_3^+]$) for either the 30 s or 6 min RI. For the 30 s RI, $[NH_3^+]$ peaked at two minutes following the tenth sprint for both CrS (196 ± 15 $\mu mol \cdot l^{-1}$; Fig. 4.3. B) and CON (172 ± 24 $\mu mol \cdot l^{-1}$; Fig. 4.3. D). For the 6 min RI,

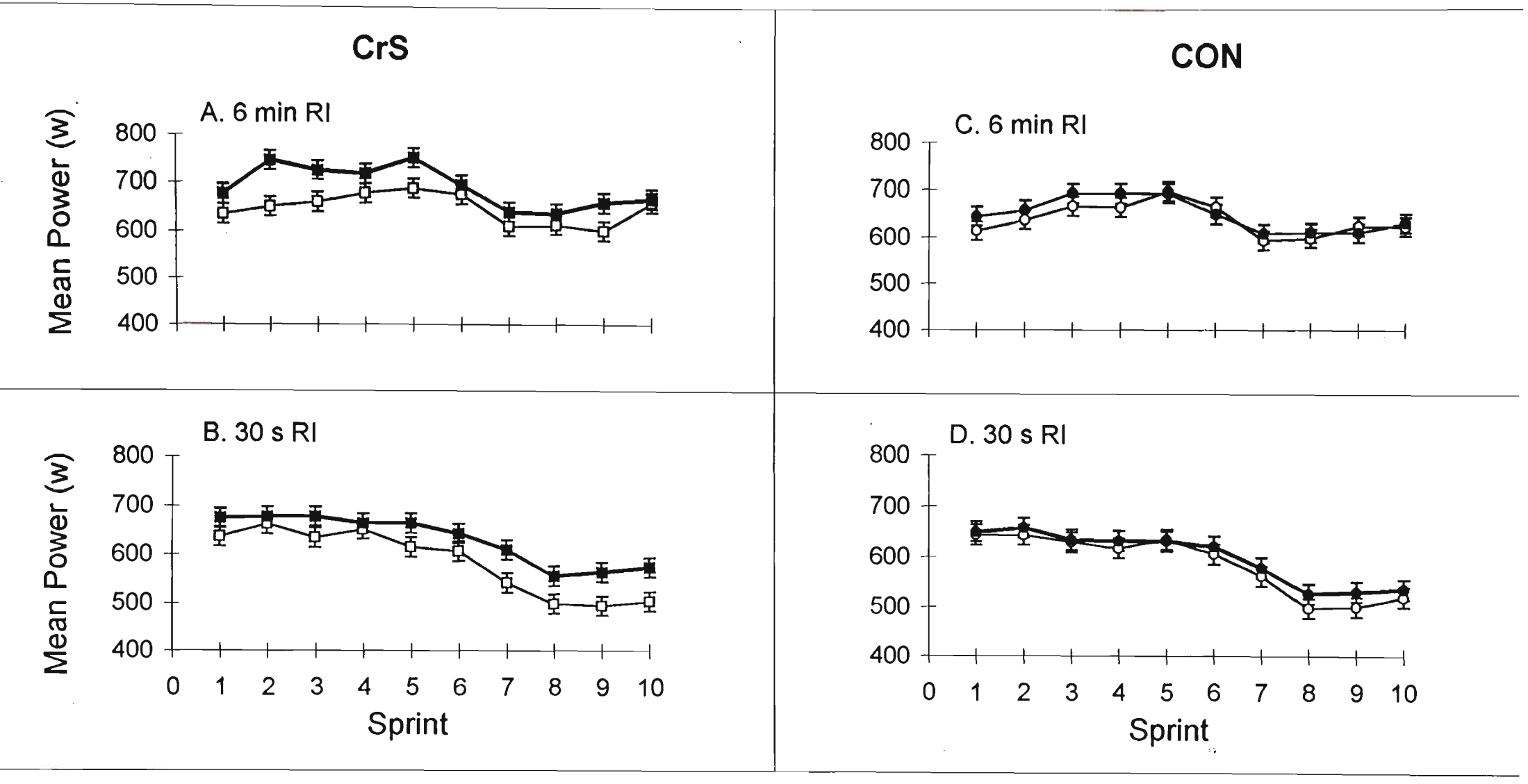


Figure 4.2 Mean Power Output (PPO) during 10 x 6 s maximal sprints, comparing post vs pre supplementation within each group for both 30 s and 6 min RI. A & B; CrS group (n = 7; mean and SEM): post (–■–) vs pre (–□–). MPO tended to be greater after Cr supplementation ($P < 0.06$). C & D; CON group (n = 7; mean and SEM): post (–●–) vs pre (–○–). MPO did not differ significantly after PL supplementation.

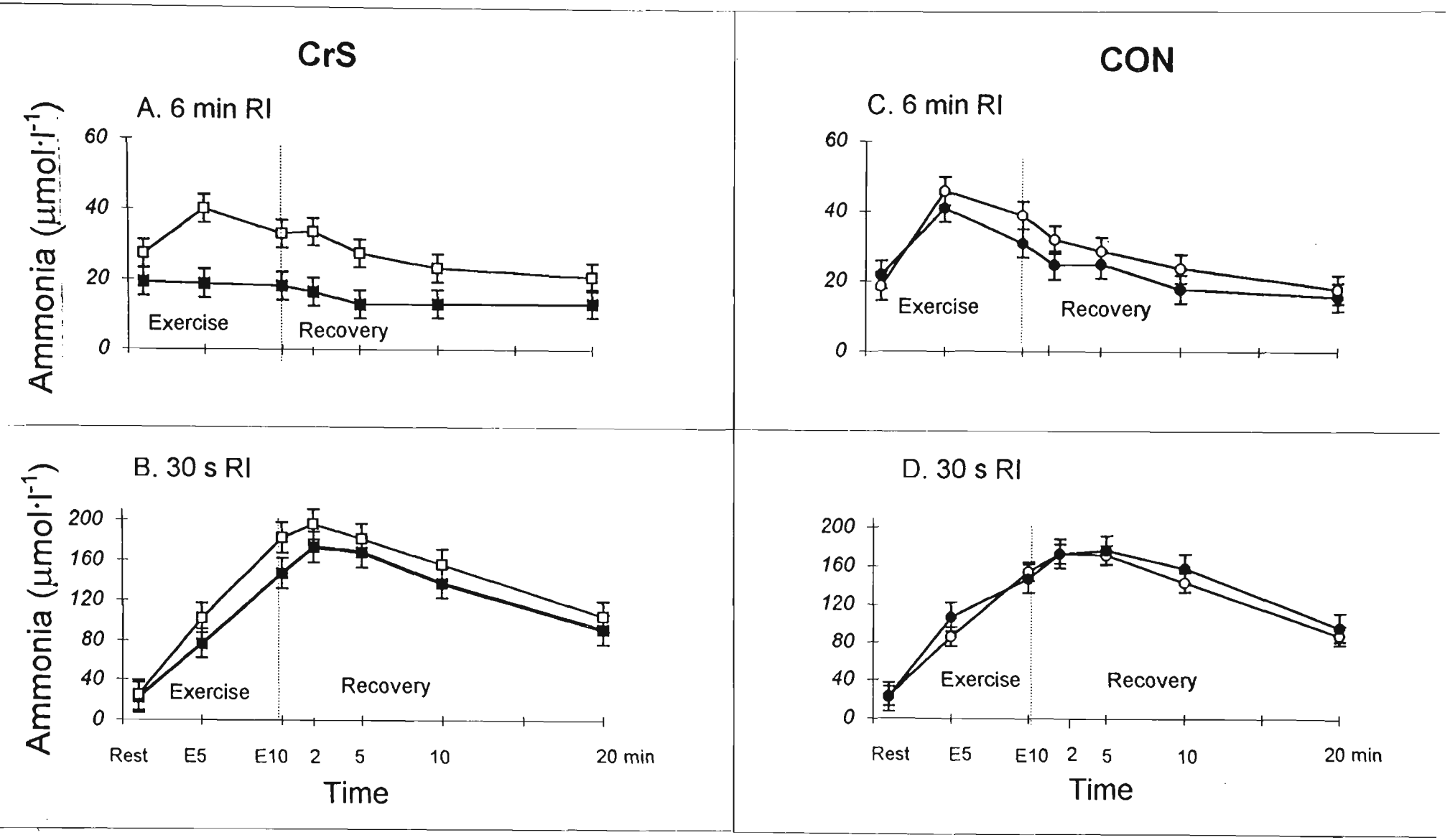


Figure 4.3 Plasma ammonia concentration ($[NH_3^+]$) obtained at rest, after exercises (E_5 & E_{10}) and during recovery (R_2, R_5, R_{10} & R_{20} min), comparing post vs pre supplementation within each group for both 30 s and 6 min RI. A & B; CrS group (n = 7; mean and SEM): post (–■–) vs pre (–□–). $[NH_3^+]$ for the 6 min RI was significantly lower after Cr supplementation ($P < 0.05$). C & D; CON group (n = 7; mean and SEM): post (–●–) vs pre (–○–).$[NH_3^+]$ did not differ significantly after PL supplementation

the highest [NH_3^+] was measured after the fifth sprint for both CrS (40 ± 4 μmol·l^{-1}; Fig. 4.3. A) and CON (46 ± 13 μmol·l^{-1}; Fig. 4.3. C). Overall, [NH_3^+] for the 30 s RI were significantly higher than for 6 min RI ($P < 0.01$).

Post-supplementation: in the CON group, there were no significant supplementation effects in [NH_3^+] (comparing post- versus pre-supplementation) for both RI (Fig. 4.3. C & D). In the CrS group for the 6 min RI, there was a significant main effect of lower [NH_3^+] after supplementation (post, 15.9 ± 3 μmol·l^{-1} versus pre, 29.2 ± 4 μmol·l^{-1}; mean ± SEM for the blood samples combined; $P < 0.05$, Fig. 4.3. A). For the 30 s RI in this group, [NH_3^+] tended to be lower following supplementation (post, 116.4 ± 14 μmol·l^{-1} versus pre, 135.1 ± 12 μmol·l^{-1}; $P = 0.07$, Fig. 4.3. B).

4.2.2 Lactate

Pre-supplementation: For both 30 s and 6 min RI, exercise resulted in significant increases in plasma lactate concentration ([La^-]) for both CrS and CON groups ($P < 0.01$, Fig. 4.3). Overall, [La^-] for the 30 s RI was significantly higher than for the 6 min RI ($P < 0.01$). There was no significant difference between the groups for [La^-] for either RI. Peak [La^-] was reached at about two minutes recovery following the tenth sprint for both CrS (17.0 ± 0.9 mmol·l^{-1}, and 7.0 ± 0.5 mmol·l^{-1} for the 30 s and 6 min RI, respectively, Fig. 4.4. A & B) and CON (16.0 ± 1.6 mmol·l^{-1}, and 7.0 ± 1.3 mmol·l^{-1} for the 30 s and 6 min RI, respectively, Fig. 4.4. C & D).

Post-supplementation: There were no significant supplementation effects for [La^-] within the CrS group for either the 30 s or 6 min RI and for the CON group for 30 s RI (Fig. 4.4.

A, B & D). However, [La^-] was significantly lower following placebo supplementation for 6 min RI ($P < 0.05$, Fig. 4.4. C). Similar to pre-supplementation, [La^-] for the 30 s RI was significantly higher than the 6 min RI for both groups ($P < 0.01$).

4.2.3 Hydrogen Ion [H^+]

For both groups, exercise resulted in greater increases in plasma [H^+] for 30 s than for 6 min RI ($P < 0.01$, Fig. 4.5. A & B; C & D). There were no significant differences between pre and post supplementation for either CrS or CON group, and no significant differences between the two groups pre-supplementation (Fig. 4.5).

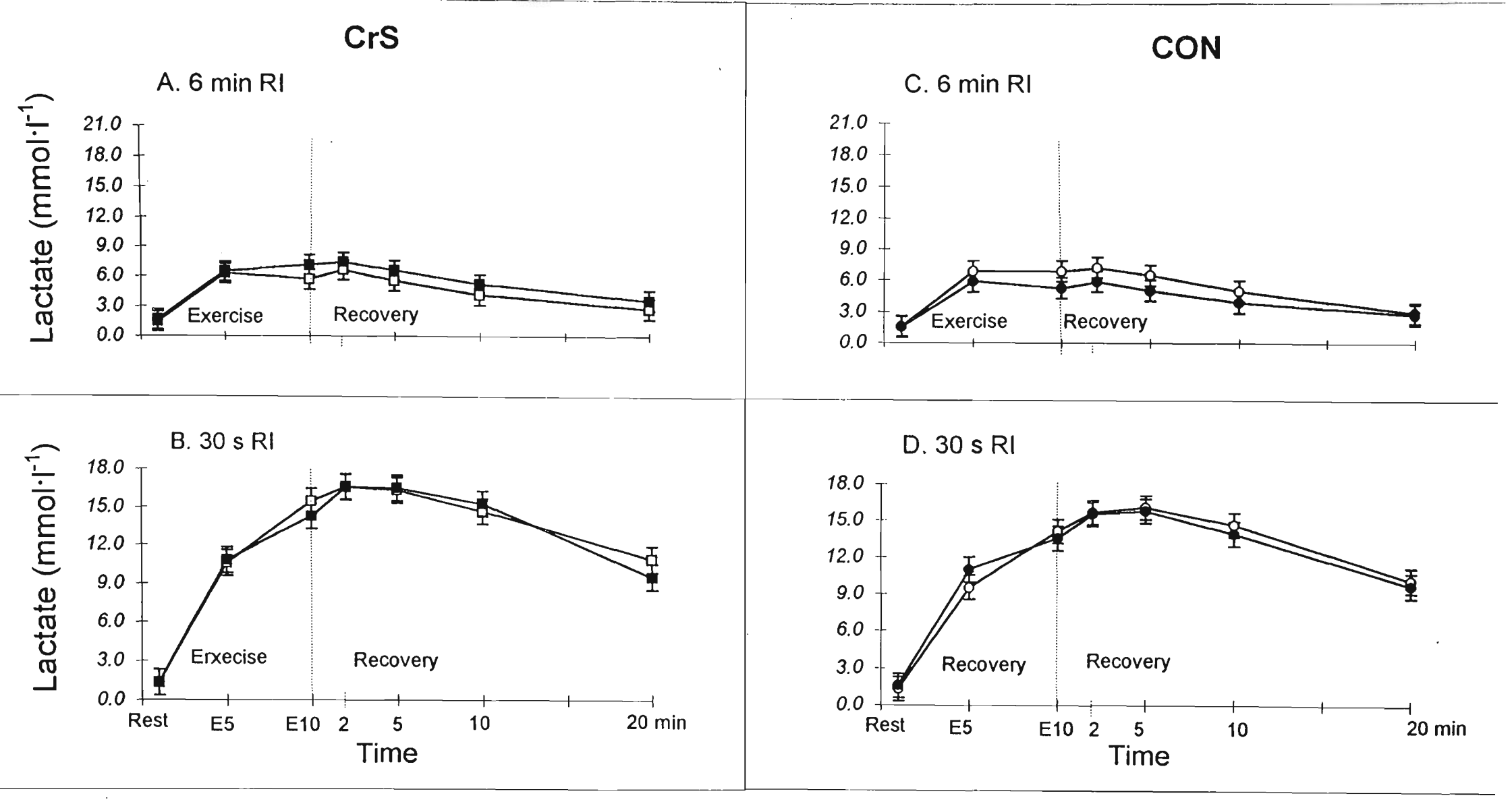


Figure 4.4 Plasma lactate concentration ([La$^-$]) obtained at rest, after exercises (E_5 & E_{10}) and during recovery (R_2, R_5, R_{10} & R_{20} min), comparing post vs pre supplementation within each group for both 30 s and 6 min RI. A & B; CrS group (n = 7; mean and SEM): post (–■–) vs pre (–□–). [La$^-$] did not differ significantly after Cr supplementation. C & D; CON group (n = 7; mean and SEM): post (–●–) vs pre (–○–).[La$^-$] for the 6 min RI was significantly lower after PL supplementation ($P < 0.05$).

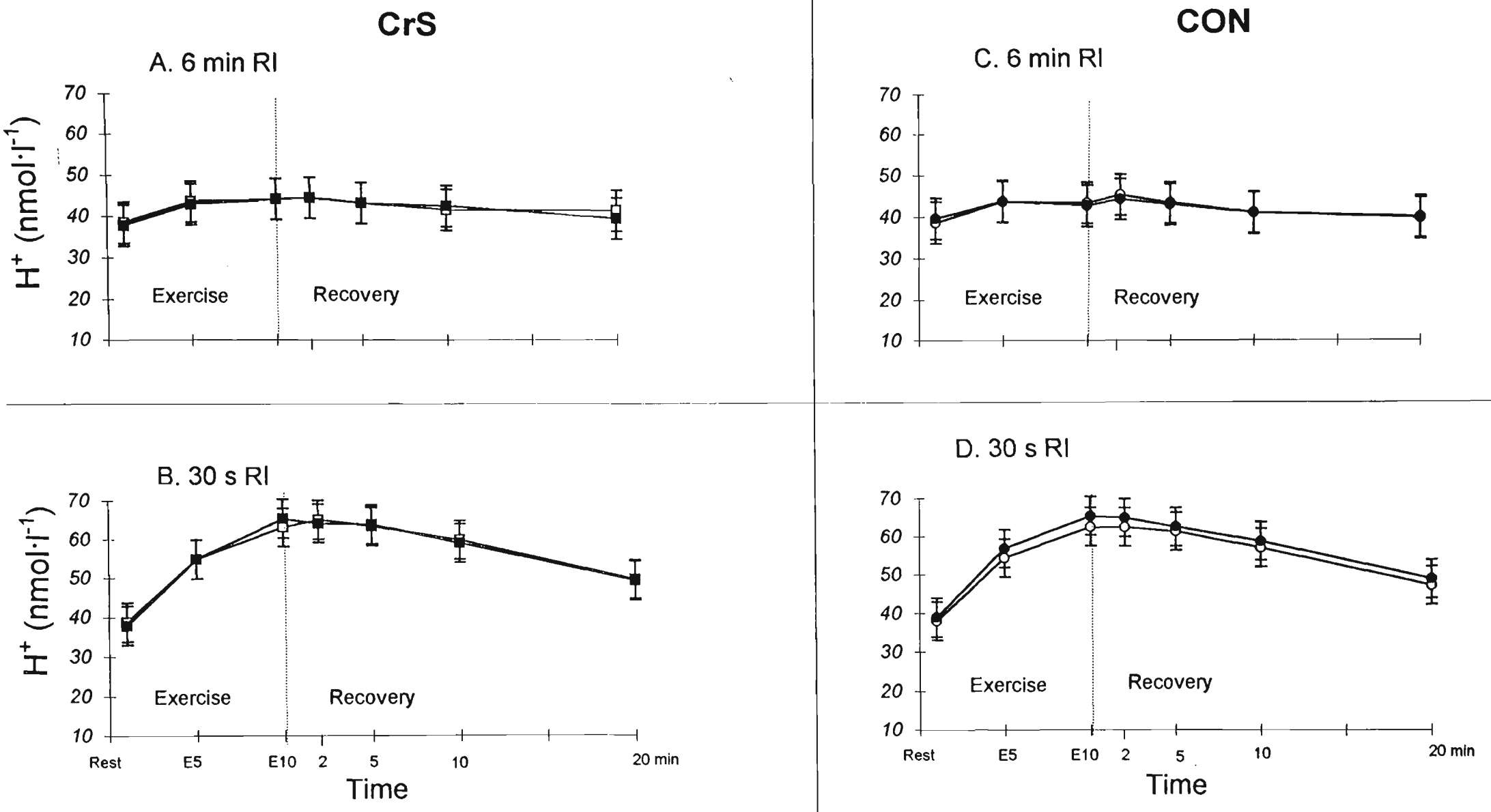


Figure 4.5 Plasma hydrogen ion concentration ($[H^+]$) obtained at rest, after exercises (E_5 & E_{10}) and during recovery (R_2, R_5, R_{10} & R_{20} min), comparing post vs pre supplementation within each group for both 30 s and 6 min RI. A & B; CrS group (n = 7; mean and SEM): post (–■–) vs pre (–□–). $[H^+]$ did not differ significantly after Cr supplementation. C & D; CON group (n = 7; mean and SEM): post (–●–) vs pre (–○–).$[H^+]$ did not differ significantly after PL supplementation.

CHAPTER 5 DISCUSSION

This study investigated the ergogenic and metabolic effects of creatine supplementation (CrS) on the performance of two series of 10 x 6 s maximal-intensity cycling sprints, one with 30 s RI, the other with 6 min RI. Following CrS, PPO and MPO increased for both RI, with the ergogenic effects being similar for the two RI. These results confirm earlier reports for brief, intermittent high-intensity exercise (BIHIX) with short (24 - 30 s) RI's (Balsom et al., 1993a; Dawson et al., 1995) but is the first study to demonstrate an ergogenic effect for BIHIX with long RI's. The practical application of this is that CrS may be an effective ergogenic aid for intermittent sports or activities where the RI between efforts is either "short" (e.g. field or court games) or "long" (e.g. track and field), or even a combination of the two. The improvements in exercise performance were associated with lower plasma $[NH_3^+]$ for both RI's, most likely due to lower adenine nucleotide degradation with CrS, whilst plasma $[La^-]$ for both RI was similar before and after CrS. The metabolic responses need to be interpreted in light of the fact that power was higher after CrS; thus the relative contributions of glycolysis and the adenylate kinase reaction to total ATP production were probably lower after CrS.

In contrast, PPO and MPO did not increase after the placebo treatment (CON) for either RI. In these experiments, plasma $[NH_3^+]$ was similar before and after treatment for both RI's, whilst there was an unexpected lowering of plasma $[La^-]$ for CON for the 6 min RI.

5.1 Ergogenic Effects of Creatine Supplementation

The ergogenic effects of CrS that occurred for both RI's in this study are in broad agreement with several previous studies using BIHIX. Most of the studies for which an ergogenic effect was described, used high-intensity, intermittent exercise, usually of a brief duration (Balsom et al., 1993a, 1995; Harris et al., 1993; Greenhaff et al., 1993, 1994; Dawson et al., 1995), with the exception of the study by Harris et al. (1993) who reported an ergogenic effect of CrS for repeated running efforts of 300 or 1000 meters with 4 and 3 min RI's, respectively. In contrast to these studies and the present one, CrS was not found to improve running performance over a six kilometer cross-country course (Balsom et al., 1993b) or performance of exercise at 120% of VO_{2max} (Balsom et al., 1993b; Febbraio et al., 1995). These latter studies were conducted at considerably lower intensities of exercise than the protocols that used BIHIX, including the present one.

The series of 10 x 6 s sprints with 30 s RI was deliberately similar to that used by Balsom et al. (1993a). Their subjects were required to cycle at a cadence of 130 $rev \cdot min^{-1}$ (EX_{130}) on one occasion and 140 $rev \cdot min^{-1}$(EX_{140}) on another separated by 24 h, random order, with each series then repeated following CrS. The EX_{130} series was designed to be intense but sustainable. The EX_{140} series was intended to cause fatigue, but in contrast to the present study where fatigue was evident from the second sprint, EX_{140} was not quite maximal. These two series enabled the researchers to study the effects of CrS on exercise metabolism during high, but constant power (EX_{130}) and on exercise performance and metabolism during a (nearly) maximal series (EX_{140}). For EX_{130}, CrS reduced plasma lactate concentration and tended to reduce plasma

hypoxanthine concentration. For EX_{140}, CrS decreased the fatigue index, as well as plasma lactate and hypoxanthine concentrations. The ergogenic and metabolic effects of CrS were attributed to increased resting levels of PCr, increased PCr resynthesis during the recovery intervals, and decreases in lactate production and adenine nucleotide degradation during sprinting, none of which was measured directly in their study (Balsom et al., 1993a). These conclusions were supported in a recent study (Balsom et al., 1995) using a similar exercise protocol, in which muscle biopsies were analysed for PCr and lactate, and blood samples for lactate and hypoxanthine. Although resting concentrations of PCr were only about 10% higher after CrS, PCr content was 50% higher immediately after the fifth of 5 x 6 s sprints with 30 s RI, suggesting a higher availability of PCr during this protocol of BIHIX. Taken together, the studies by Balsom et al. (1993a, 1995) and the present study, strongly suggest that CrS is an effective ergogenic aid for BIHIX, whether the work is performed at maximal or near-maximal intensity.

In previous studies which investigated the effects of CrS on performance of BIHIX, only short RI's (30 s RI) were applied (Balsom et al., 1993; Greenhaff et al., 1993; Dawson et al., 1995). There have been no studies comparing the effects of short versus long RI's. In the present study with CrS, 30 s and 6 min RI were utilised for 10 x 6 s maximal cycling sprints in order to describe the types of RI (and therefore, activities) for which CrS is effective. In contrast to this study, previous research on the effects of CrS for intermittent exercise using "long" RI's (ie at least 1 min), were for exercise bouts of at least 30 s duration (Greenhaff et al., 1993; Greenhaff et al., 1994b; Harris et al., 1993; Febbraio et al., 1995; Green et al., 1993). In the case of all these studies, the exercise intensity was necessarily lower than in the present one, with an

upper limit of about 130% of VO_{2max}. Therefore this is the first study to demonstrate ergogenic effects of CrS for repeated maximal sprinting with RI's that were long enough to enable PCr resynthesis to be nearly complete.

The exercise performance data reported in this thesis indicate that the ergogenic effect of CrS does not depend on the length of the RI because there was no significant interaction between RI and pre- versus post-treatment for CrS for either PPO and MPO. Since it can be assumed that PCr resynthesis was almost complete for the 6 min RI, but less than 50% for the 30 s RI (Bogdanis et al., 1996), this suggests that CrS may have an ergogenic effect whether or not PCr resynthesis is complete between sprints.

5.2 Effects of CrS on Metabolism during High-Intensity Exercise

Several inter-related metabolic mechanisms have been proposed to explain the ergogenic effects of CrS during intermittent high-intensity exercise. These include increases in total creatine, PCr and Cr_f contents at rest (Harris et al., 1992; Balsom et al., 1995; Febbraio et al., 1995), PCr utilisation during exercise (Greenhaff et al., 1994a), PCr resynthesis during recovery (Greenhaff et al., 1994a), muscle buffering capacity during exercise (Greenhaff et al., 1994a), and reductions in adenine nucleotide degradation (Balsom et al., 1993a) as well as lactate production (Balsom et al., 1993a).

In relation to these, the series of 10 x 6 s maximal intensity sprints with 30 s RI was chosen because it has recently been shown for this sprint protocol that PCr availability

is very important to the production of ATP during sprinting, but PCr resynthesis is incomplete (≤ 50%) after 30 s RI (Gaitanos et al., 1993). Therefore it was expected that CrS would be effective for this series of sprints. The 6 min RI was chosen as a contrast to the 30 s RI on the assumption that PCr resynthesis would be almost complete for the former, but incomplete for the latter (Hultman et al., 1967; Bogdanis et al., 1995).

5.2.1 Effects of CrS on Adenine Nucleotide Degradation

The increases in plasma concentration of [NH_3^+] reported in response to intense exercise are usually interpreted to indicate increased adenine nucleotide degradation, rather than amino acid deamination, since these are accompanied by rises in muscle concentrations of IMP (Graham et al., 1990; Katz et al., 1986b; Stathis et al., 1994) and plasma concentrations of the purines, hypoxanthine and uric acid (Balsom et al., 1992a). In the present study, plasma [NH_3^+] tended to be lower after CrS for the 30 s RI; at the same time PPO and MPO were higher. This concurs with the studies of CrS by Balsom et al. (1993a), who reported lower plasma hypoxanthine accumulation for a very similar sprint protocol to that used here, and Greenhaff et al. (1993) and Birch et al. (1994) who each reported lower plasma [NH_3^+] in response to maximal voluntary contractions and a series of 30 s sprints, respectively. These data suggest that CrS was associated with lower rates of adenine nucleotide degradation for this series of BIHIX, probably by increasing PCr availability during sprinting (Greenhaff et al., 1994a; Balsom et al., 1995). These findings were taken to indicate that there was less reliance on the adenylate kinase reaction to maintain ATP production following CrS, leading to lower rates of production of AMP and IMP. In contrast, Balsom et al. (1995) did not find decreases in plasma hypoxanthine accumulation with CrS following a series of

submaximal 6 s sprints followed by a 10 s maximal sprint. However hypoxanthine "accumulation" was derived from a single blood sample taken 15 min post-exercise and so it is possible that the peaks in plasma hypoxanthine concentration were not detected. In two studies using much lower exercise intensities, there were no significant changes after CrS in ammonia concentrations in muscle (Febbraio et al., 1995) or plasma (Balsom et al., 1993b).

It was surprising that for the series of 10 x 6 s sprints with 6 min RI (with a work: rest ratio of 1:60), plasma [NH_3^+] had risen to approximately double the resting concentration following the fifth of the ten 6 s sprints for the two placebo (CON) trials and the CrS group prior to supplementation, although these rises were much lower than for 30 s RI. Interestingly, CrS was also associated with lower plasma [NH_3^+] for 6 min RI; during this series of sprints and during recovery, [NH_3^+] remained virtually at the pre-exercise level. These results suggest that without CrS, there was some loss from the adenine nucleotide pool, possibly due to incomplete PCr resynthesis during these long rest intervals (Bogdanis et al., 1995), but that this loss was eliminated by CrS, perhaps due to increased PCr availability after CrS (Greenhaff et al., 1994a; Balsom et al., 1995).

5.2.2 Effects of CrS on Glycolysis

No difference was found in plasma [La^-] in the CrS group for both RI (post versus pre-CrS), despite the greater amount of work performed after CrS. This finding is in general agreement with previous research which reported increased work and/or power after CrS, but no significant changes in blood [La^-] (Greenhaff et al., 1993; Birch et al., 1993; Earnest et al., 1994). One study found an increase in blood [La^-]

following CrS (Dawson et al., 1995) corresponding to higher power, but blood [La^-] also rose in the placebo subjects following supplementation and there was no significant difference between the two groups. Together, these studies indicate that glycolysis was not higher after CrS, despite the higher work, and it is reasonable to infer that lactate concentration at given workloads would have been lower following CrS. This was supported by the findings of lower blood [La^-] with CrS during fixed, high-intensity exercise (Balsom et al., 1993a).

However all of these studies, including the present one, were limited by the use of blood rather than muscle lactate analyses, and furthermore, neither lactate efflux from muscle nor lactate clearance from blood were accounted for. Nevertheless, recent support for the proposal was provided by Balsom et al. (1995) who reported lower muscle [La^-] following a series of 5 x 6 s high-, constant-intensity sprints with 30 s RI, and no significant difference in lactate concentration for a sixth maximal sprint where the power was greater following CrS. In contrast, Greenhaff et al. (1994a) reported higher muscle [La^-] after CrS immediately following a given intensity of electrically-evoked muscle contractions. This did not concur with their other published findings (Greenhaff et al., 1993, 1994b) as discussed earlier (see Literature Review).

In the present study, it is difficult to interpret the lower [La^-] that were observed for CON following supplementation for 6 min RI, because PPO and MPO were not significantly different for this group. This did not occur for the 30 s RI in this group. Two possibilities are that a training effect occurred during the study, even though the subjects were requested to maintain a consistent training regime, or a psycho-physiological influence of placebo treatment. The latter is unlikely as this influence would then be expected for the CrS group and for the 30 s RI in the CON group.

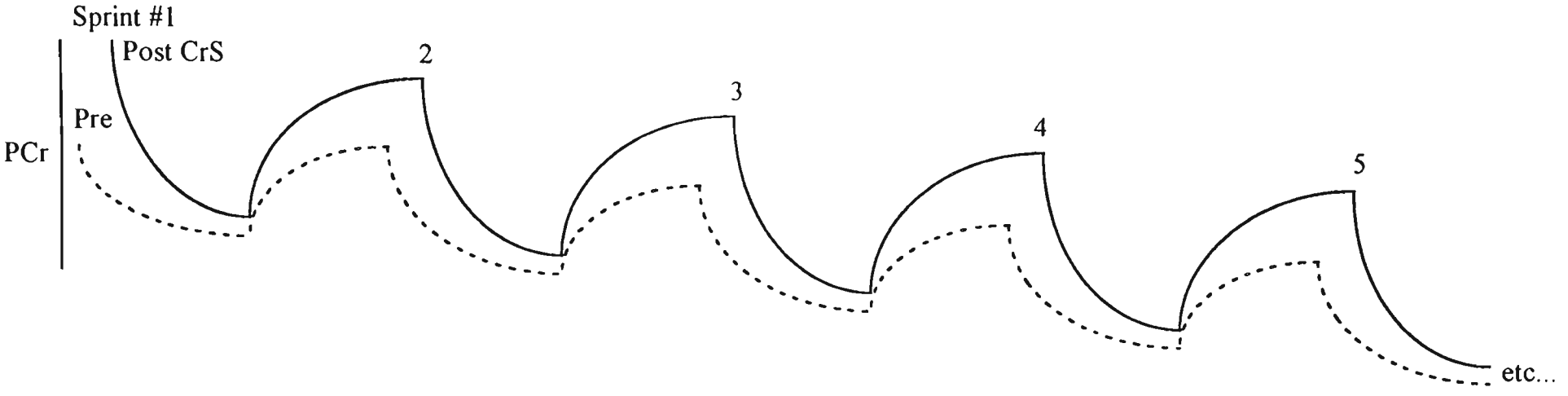
5.2.3 Effects of CrS on Oxidative Phosphorylation

The role of oxidative phosphorylation in the exercise responses reported in this thesis should not be discounted. Using the same series of sprints (10 x 6 s with 30 s RI), Gaitanos et al. (1993) provided evidence for an increasing contribution from aerobic glycolysis during the latter part of the series. The interaction between this and CrS is not well understood, with only one study of oxygen consumption during BIHIX (Balsom et al., 1993). They reported that $\dot{V}O_2$ was lower for the EX_{130} series of sprints, and unchanged for the EX_{140} series after CrS. These findings are surprising, given that PCr resynthesis is dependent on oxygen delivery to muscle (Sahlin et al., 1979) and that PCr resynthesis during recovery from intense exercise is greater following CrS (Greenhaff et al., 1994a; Balsom et al., 1995). Perhaps other metabolic processes were downregulated in these exercise responses, such as glycolysis due to the lower concentrations of ADP, AMP and H^+ that probably occurred in these responses (Balsom et al., 1995). In the present study, CrS had a major effect on PPO but lesser on MPO, suggesting that glycolysis and oxidative phosphorylation may be impaired by CrS. Since feeding in rats of a creatine analogue, beta-guanidinoproprionic acid, increased mitochondrial enzyme activity and reduced glycolytic enzyme activity (Shoubridge et al., 1985). However, this section discussed about effects of CrS on oxidative phosphorylation in human only, more research is needed on $\dot{V}O_2$ kinetics and related metabolic processes with CrS.

5.3 Proposed Mechanism to explain the Exercise Responses Reported in this Thesis

Data reported in the literature and the present results indicate that the ergogenic effect of CrS during brief, intermittent, high-intensity exercise is likely to be attributed to higher PCr availability. This is due to greater PCr hydrolysis during sprinting and PCr resynthesis during recovery periods. A hypothetical model based on enhanced PCr availability for both the 30 s and 6 min RI series is given in Figure. 5.1 & 5.2. Adenosine nucleotides appear to be conserved better with CrS. The ergogenic effect of CrS does not appear to be due to an increase in ATP production from glycolysis. The contribution from oxidative phosphorylation is unclear.

30 s rest interval series

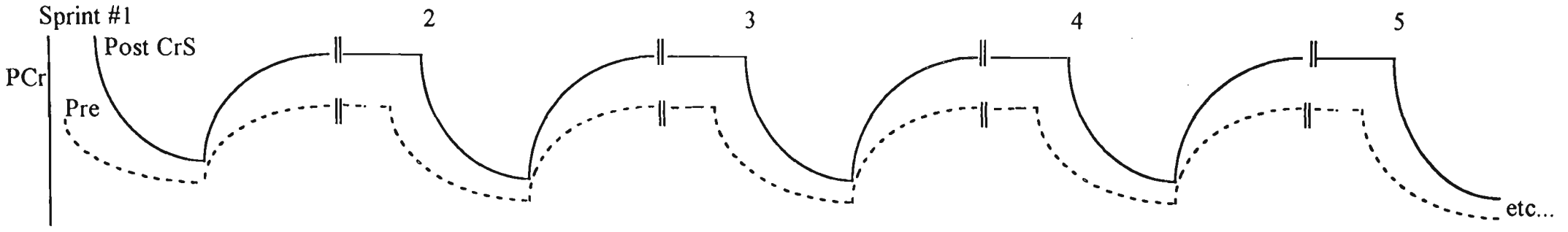


For 30 s rest intervals (RI) CrS group probably underwent:

a. Incomplete PCr repletion during RI
b. Post CrS ↑ [PCr] before each sprint (1-10)
c. Higher PCr repletion rate for post CrS than pre CrS during RI
d. Post CrS ↑ capacity of creatine kinase system resulting in greater depletion of PCr in each sprint ⇒ ↑ ATP resynthesis & ↓ IMP & $[NH_3^+]$

Figure. 5.1 The proposed mechanism for the ergogenic effects of creatine supplementation (CrS) on exercise performance during 30 s RI series. Pre (---) vs post CrS (—).

6 min rest interval series



For 6 min rest intervals (RI) CrS group probably underwent:

a. Almost complete PCr repletion during RI
b. Post CrS ↑ [PCr] before each sprint (1-10)
c. Higher PCr repletion rate for post CrS than pre CrS during RI
d. Post CrS ↑ capacity of creatine kinase system resulting in greater depletion of PCr in each sprint ⇒ ↑ ATP resynthesis & ↓ IMP & $[NH_3^+]$

Figure. 5.2 The proposed mechanism for the ergogenic effects of creatine supplementation (CrS) on exercise performance during 6 min RI series. Pre (---) vs post CrS (—).

CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

6.1 Conclusions

The present study demonstrated that after a period of a high dose of creatine feeding (30g of creatine per day for 5 days), peak power was increased and mean power tended to be higher for both 30 s RI and 6 min RI during 10 x 6 s cycling sprints. There were no increases in work or power for the group of subjects that were fed the placebo.

The ergogenic effects were not significantly greater for the 30 s RI than for the 6 min RI, as was hypothesised. This suggests that the actual amount of PCr repletion that occurs during the recovery intervals is not critical to the effects of CrS. Peak power declined progressively during the sprint series with the 30 s RI, but was maintained at near constant levels during the 10 sprints with the 6 min RI.

Plasma ammonia concentrations were lower following CrS for both RI, in spite of more work being performed during exercise after CrS. These data suggest that the ergogenic effect of CrS may be partly attributed to reduced degradation of adenine nucleotides, and greater ATP turnover, caused by an increase in the pre-exercise PCr stores.

No change was evident in the post-exercise plasma lactate concentrations when comparing the values before and after CrS for both 30 s and 6 min RI sprint series. This observation suggests that CrS may have no direct effect on glycolysis during this

exercise, the likely reason for these increases in peak and mean power is probably due to a change from sources of energy production other than glycolysis.

6.2 Recommendations For Further Research

No studies have measured PCr resynthesis with and without creatine supplementation for BIHIX. The only study that measured the effects of CrS on PCr resynthesis (Greenhaff et al., 1994) used electrically-evoked muscle contractions with a total contraction time of 32 s and a work: rest ratio of 1:1. The 10 x 6 s with 30 s RI exercise protocol used in the present study is not unlike that found in many competitive sports. It is important to study the effects of CrS on PCr resynthesis for this protocol, given that several investigators are now suggesting that increased PCr resynthesis is a major metabolic mechanism. At the same time, it may be able to determine the effects of CrS on PCr utilisation for the sprint series by performing biopsies immediately before and immediately after selected sprints in the series, similar to the approach by Gaitanos et al. (1993) in their study without CrS.

The importance of oxidative phosphorylation in the latter part of a series of should not be discounted. Using the same series of sprints (10 x 6 s with 30 s RI), Gaitanos et al. (1993) provided evidence for an increasing contribution from aerobic glycolysis during the last few sprints. The interaction between this and CrS is not well understood, with only one study of oxygen consumption during brief, intermittent high-intensity exercise (Balsom et al., 1993), and that was for whole body $\dot{V}O_2$. They reported that $\dot{V}O_2$ was lower for both series of sprints (EX_{130} and EX_{140}; see Table 2.3) after CrS. This was both surprising and difficult to interpret. More research is needed on $\dot{V}O_2$ kinetics, particularly at the level of active muscle, and related metabolic processes with CrS.

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APPENDIX A

SUBJECT INFORMATION, INFORMED CONSENT STATEMENTS AND VO_2peak TEST

VICTORIA UNIVERSITY OF TECHNOLOGY

STANDARD CONSENT FORM FOR SUBJECTS INVOLVED IN EXPERIMENTS

CERTIFICATION BY SUBJECT

I, ...

of.....
certify that I have the legal ability to give valid consent and that I am voluntarily giving my consent to participate in the experiment entitled :

"The Effects of Creatine Supplementation on Performance of High-Intensity Intermittent Exercise"

being conducted at Victoria University of Technology by :

Dr. Steve Selig, Mr. Binh Chu Ba, Dr. Michael J. McKenna, Professor David Lawson.

I certify that the objectives of the experiment, together with any risks to me associated with the procedures listed hereunder to be carried out in the experiment, have been fully explained to me by :

Dr.Steve Selig

and that I freely consent to participation involving the use on me of these procedures.

Procedures

VO_2peak test.
60 seconds cycle sprint test.
10 x 6 seconds cycle sprint tests.
Creatine supplementation.
Venous cannulation.

I certify that I have had the opportunity to have my questions answered and that I understand that I can withdraw from the experiment at any time and that this withdrawal will not jeopardise me in any way.

I have been informed that the confidentiality of the information I provide will be safeguarded.

Signed : .....)
)
Witness other than the experimenter :) Date : .....
)
.....)

VICTORIA UNIVERSITY OF TECHNOLOGY

Subject Information Sheet

The tittle:
The Effects of Creatine Supplementation on Performance of High-Intensity Intermittent Exercise

INVESTIGATORS:
Dr Steve Selig, Mr Binh Chu Ba, Dr Michael McKenna, Professor David Lawson.
Department of Physical Education and Recreation, Victoria University of Technology.

Aim of the study:
This study will attempt to determine whether creatine, a dietary supplement normally present in meat, will enhance your ability to perform maximal intermittent exercise and change your muscle energy stores.

Subject participation:
As a volunteer, you are free to withdraw from the study at any time, without any adverse effects or reactions.

Exercise Testing procedures:

Note: there are no muscle biopsies taken in this study!! The plan of the study is tabled below (Table 1). On five occasions, you will be required to attend the Exercise Physiology Laboratory (Room L329, Building L), at the Footscray Campus of Victoria University of Technology. This study is proposed to investigate the effect of Cr supplementation on the performance of 10 x 6-seconds " all out " cycling exercise, interspersed with rest intervals of 30 seconds on one day and 6 minutes rest on another day. The order of the tests (ie 30 seconds rest intervals or 6 minute rest intervals) will be randomised throughout the study. You will initially undergo a $\dot{V}O_2peak$ test. One week later, you will undergo one of the sets of sprints. Two days later, the other set of sprints will be conducted. The 16 subjects will then be divided into two matched groups (creatine supplementation group or placebo group) on the basis of the summation of the work accomplished during the two series. Both researchers and subjects will be "blinded" as to the group allocations (ie not know which treatment the subjects are assigned to). Following this and after a further one week of rest, you will undergo five days of the treatment (ie creatine or placebo) that you were allocated to. You will then perform the two series of sprints (in the same order as on the first occasion) with a rest period of two days in between. Blood samples will only be taken during the four series of 10 x 6 seconds of sprint exercise (ie not during the $\dot{V}O_2peak$). These samples will be taken at rest, immediately following the fifth and tenth sprint repetitions, as well as at two, five, ten and twenty minutes after the final sprint repetition.
Note: the order of the two series of sprints (ie 30 second or 6 minute rest intervals) will be randomised for each group.

Blood samples:

Prior to the test, you will have a catheter inserted into a superficial vein in the forearm. The "flashback" sign will be used to indicate successful entry into the blood vessel, upon which the needle will be withdrawn immediately and a small plastic tube with a small "tap" will be connected to the catheter for ease of sampling. The catheter entry point will be covered by "second skin" throughout the experiment. Once the catheter is in place, it is a simple and painless procedure to draw blood samples. This will allow us to measure some of the changes in the blood that happen in response to the exercise. Between blood samples, the catheter will be filled with sterile salt water (similar to your own blood plasma); this will contain a very small amount of anti-coagulant which will prevent the catheter from clotting. The total volume of blood to be taken on any one day *will not exceed 200 ml* (but will more likely be not much more than 100 ml) which represents less than half of the volume taken in a blood donation (450 ml) and is equal to about 4% your blood volume. There is a separate consent form for this procedure.

Creatine Supplementation procedure:

There are no reported negative side effects of creatine supplementation. The dose used in this study has been used previously with no ill effects. You will be issued with several bottles of fluid supplement. You will be required to consume two hundred (200) ml of the supplement, six (6) times per day for three (3) days prior to the exercise performance test. The recommended times for drinking the supplement are (1) with breakfast, (2) mid-morning, (3) with lunch, (4) mid-afternoon, (5) at dinner time and (6) later in the evening. Half of the group will be provided with creatine supplement in a powder form. The other half of the group will be provided with a powder consisting of a placebo substance. When you make up the drinks, it will be impossible to determine which you have been randomly assigned.

By signing the informed consent form you are indicating that the tests and procedures have been explained to you and are understood by you. Also, it is accepted by the investigators and by yourself that you are participating voluntarily in the study and that you are free to withdraw from the investigation at any time. Thank you for your co-operation.

VICTORIA UNIVERSITY OF TECHNOLOGY
Exercise Test Appointment Sheet for the study

"The Effects of Creatine Supplementation on Performance of High-Intensity Intermittent Exercise"

NAME: «title» «firstname» «lastname»

TYPE OF EXERCISE TEST: **«TEST»**

DATE OF APPOINTMENT: «date»

TIME OF APPOINTMENT: «time»

PLACE OF APPOINTMENT: Room «place»

Building L is located on the **Footscray** campus of the university; park in the carpark opposite the campus on Ballarat Road (near the corner of Geelong Road); the building is located near the city end of the campus, behind the new red brick building. When you arrive, I'll give you a visitor's carpark pass if you need one. If you receive a parking infringement notice (from VUT!!), send it to me and I will have it cancelled.

PHONE NO: (03) 688 4421 (Dr. Steve Selig)
FAX: (03) 688 4891

INSTRUCTIONS:

1. Do not exercise on the day of the test.

2. If exercising on the day before the test, then make it light exercise.

3. Eat a light meal 2-3 hours prior to the test, or as directed.

4. Bring running shoes and shorts, or tracksuit.

5. Females wear bikini top or sports bra. Wear a T-shirt over the top.

6. Change and shower facilities are available (bring towel.)

7. Bring any other papers that have been sent to you, and ensure that you have supplied the information where indicated and signed the forms.

8. Car parking: see above

9. Other instructions: .....

$\dot{V}O_2$peak Test

All subjects completed an incremental exercise test on a mechanically-braked cycle ergometer (Monark Ergogenics 868, Varberg, Sweden) to determine $\dot{V}O_2$peak. Subjects commenced cycling at 40 W (@ 80 rev.min^{-1}) for 4 min followed by three min of each of 80, 100, 120 and 160W. Power output was then incremented by 20 W.min^{-1} by increasing the pedal cadence until volitional exhaustion to determine $\dot{V}O_{2peak}$. Metabolic measurements were made using an expired gas mixing chamber system, where expired gas was directed through a low-resistance valve (Hans Rudolph 2-way non-rebreathing); expired ventilation was determined by a spirometer (Pneumoscan S30) and analysed for oxygen (Applied Electrochemistry S-3A O_2) and carbon dioxide (Applied Electrochemistry CD-3A CO_2; Ametek, PA. USA). The gas analysers were calibrated before and after the test using standard gases of known concentration. Oxygen consumption (VO_2), carbon dioxide output (VCO_2), respiratory exchange ratio (RER) and ventilatory equivalents for both VO_2 and VCO_2 were determined every 15 s using standard algorithms by an IBM compatible computer (software-VACUMED, KL engineering, California, USA). VO_2peak was defined as the highest oxygen consumption during a 15 s interval at the peak workrate. Heart rate was monitored throughout by electrocardiography (X- SCRIBE ™; Mortara, Milwaukee, USA).

Dear Prospective Subject for a study entitled

"The Effects of Creatine Supplementation on Performance of High-Intensity Intermittent Exercise"

I have attached the subject information sheets (2 pages) which are for you to read and keep. If you have any questions regarding any aspect of the study, please ring me on

688 4421 (work: up to 23 Dec and after the 16 Jan)
or
578 7226 (home: first two weeks in January)

****Note: if you decide to participate in the study, then **fill in all of the other sheets and return them to me using the address label supplied**.
Remember to sign and have your signature witnessed in the relevant spaces.
***Note: even if you agree to participate and sign all the relevant papers, you are still **free to withdraw** from the study at **any time** .... even if the study has started!!!

I hope that you will participate in the study. Merry Xmas!

Dr.Steve Selig

APPENDIX B

TYPICAL STANDARD CURVES FOR PLASMA METABOLITES

METHOD OF ANALYSIS

PLASMA AMMONIA

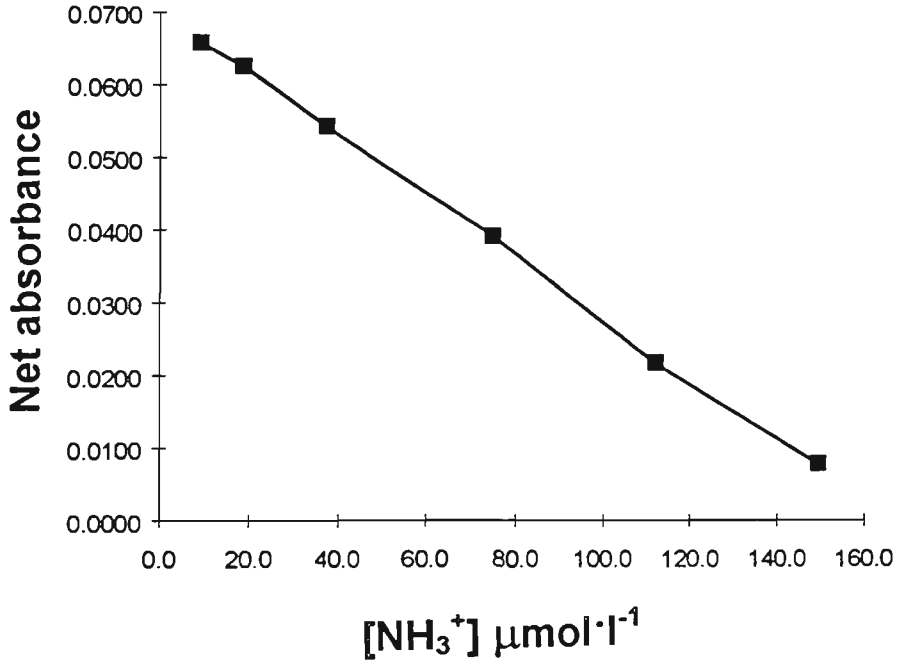

Figure B.1 A typical standard curve for plasma ammonia concentration analysis

PLASMA LACTATE

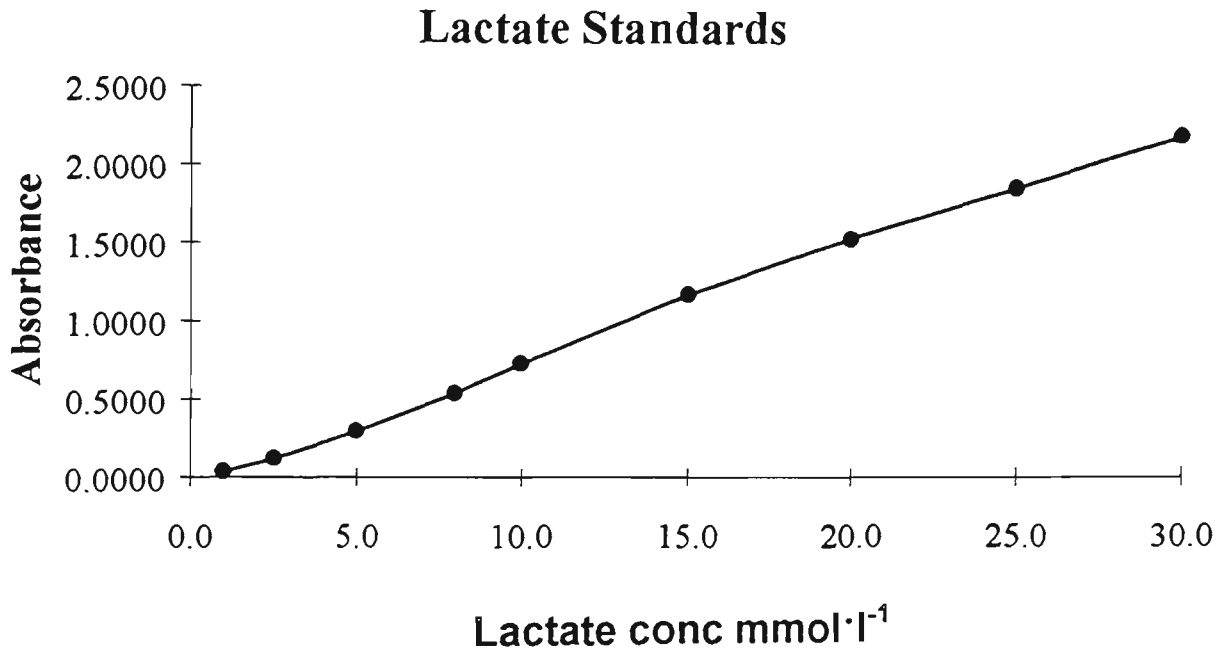

Figure B.2 A typical standard curve for plasma lactate analysis

APPENDIX C

RAW DATA

Table C.1 Subject Physical Characteristics

CrS GROUP (creatine supplementation)

SUBJECT	AGE (years)	BODY MASS (kg)	HEIGHT (cm)	VO_2 max(kg)
MC	21	77.2	179.0	66.97
JM	21	63.3	177.0	60.65
LBC	19	75.2	178.0	58.54
NW	18	77.7	181.0	50.46
JS	22	68.4	171.0	54.61
DL	18	75.8	164.0	69.81
ML	27	78.0	169.0	58.10
n	7	7	7	7
MEAN	20.9	73.7	174.1	59.9
SEM	0.6	2.4	1.4	2.4

CON GROUP (placebo supplementation)

SUBJECT	AGE (years)	BODY MASS (kg)	HEIGHT (cm)	VO_2 max(kg)
BS	22	81.6	180.0	58.22
DM	20	57.0	167.0	64.64
PW	20	82.0	184.0	51.79
MB	25	74.0	183.0	71.08
BB	17	75.4	177.0	47.63
DF	25	71.7	175.5	56.20
TS	19	75.3	170.0	58.90
n	7	7	7	7
average	21.5	73.6	177.8	58.3
SEM	1.2	3.5	2.4	3.2

PEAK POWER OUTPUT (W)

A. 30 s Rest Interval Series

Subject	Sprint # 1	2	3	4	5	6	7	8	9	10
CrS Group										
Pre Supplementation										
MC	990	920	929	901	878	805	761	734	714	737
JM	827	860	841	901	849	818	803	852	846	835
LBC	835	842	870	862	856	782	776	799	794	791
NW	1054	1029	978	1006	947	909	902	879	849	925
JS	846	839	743	765	712	726	720	659	664	675
DL	911	924	910	831	782	769	742	640	594	625
ML	901	901	886	875	854	812	754	753	779	772
n	7	7	7	7	7	7	7	7	7	7
Mean	909	902	880	877	840	803	780	759	749	766
SEM	32	25	28	28	28	21	23	34	36	38
Post Supplementation										
MC	1120	1100	1094	1037	967	958	951	916	908	893
JM	881	894	898	888	796	842	816	851	831	842
LBC	921	895	906	908	874	885	873	830	838	867
NW	1187	1163	1171	1128	1106	1029	983	961	1050	969
JS	872	809	788	733	783	760	738	810	775	770
DL	950	880	845	870	897	853	801	768	792	873
ML	866	902	904	893	861	824	787	772	793	779
n	7	7	7	7	7	7	7	7	7	7
Mean	971	949	944	922	898	879	850	844	855	856
SEM	50	53	56	52	45	36	35	27	38	25
CON Group										
Pre Supplementation										
BS	1175	1116	1082	1052	987	925	915	874	877	795
DM	813	792	782	768	749	673	634	640	618	610
PW	1022	977	971	939	909	879	861	874	874	855
MB	865	881	837	860	910	895	861	887	866	866
BB	869	870	879	865	842	749	725	749	729	818
DF	732	751	722	756	730	712	708	701	716	733
TS	915	893	887	805	817	762	738	790	797	796
n	7	7	7	7	7	7	7	7	7	7
Mean	913	897	880	864	849	799	777	788	782	782
SEM	55	46	45	39	35	37	39	36	37	33
Post Supplementation										
BS	1166	1162	1063	1036	919	890	885	793	781	823
DM	856	809	726	707	711	660	619	680	659	691
PW	1021	980	939	904	904	906	905	901	887	888
MB	912	890	897	902	936	907	911	914	903	908
BB	835	887	854	817	820	810	794	770	749	798
DF	800	794	779	757	748	745	740	720	728	708
TS	905	890	899	812	835	787	792	789	819	764
n	7	7	7	7	7	7	7	7	7	7
Mean	928	916	880	848	839	815	807	795	789	797
SEM	52	51	45	45	36	38	44	36	36	34

PEAK POWER OUTPUT (W)

B. 6 min Rest Interval Series

	Sprint #									
Subject	1	2	3	4	5	6	7	8	9	10
CrS Group										
Pre Supplementation										
MC	1084	1048	1012	1072	1069	1052	1008	1018	1029	1064
JM	791	816	823	822	810	818	807	815	808	808
LBC	882	894	899	902	870	895	916	921	915	908
NW	954	950	923	971	928	936	892	912	837	917
JS	827	845	833	836	863	814	819	773	788	822
DL	977	956	903	883	907	860	882	914	903	985
ML	869	892	883	904	902	905	908	917	896	917
n	7	7	7	7	7	7	7	7	7	7
Mean	912	914	897	913	907	897	890	896	882	917
SEM	38	29	24	32	31	31	25	30	31	34
Post Supplementation										
MC	1093	1115	1152	1137	1187	1140	1167	1169	1159	1181
JM	900	920	892	893	882	894	885	901	923	918
LBC	937	917	938	943	951	927	911	931	917	921
NW	1136	1156	1147	1127	1102	1023	1063	1041	1025	989
JS	798	743	798	774	797	772	759	737	734	786
DL	1019	1074	1053	987	952	980	968	991	986	1007
ML	916	916	888	891	942	938	922	918	909	931
n	7	7	7	7	7	7	7	7	7	7
Mean	971	977	981	965	973	953	954	955	950	962
SEM	48	59	54	53	54	47	54	55	53	49
CON Group										
Pre Supplementation										
BS	1178	1127	1113	1122	1167	1136	1180	1135	1156	1191
DM	701	691	685	651	666	639	666	690	709	687
PW	912	966	989	989	1004	999	1020	997	987	975
MB	905	901	896	884	935	901	893	882	899	894
BB	837	851	813	829	880	879	907	925	905	911
DF	746	745	754	755	752	744	720	741	749	761
TS	947	980	971	954	972	993	949	902	999	979
n	7	7	7	7	7	7	7	7	7	7
Mean	889	894	889	883	911	899	905	896	915	914
SEM	59	56	56	59	63	63	66	57	58	62
Post Supplementation										
BS	1163	1171	1153	1169	1151	1151	1129	1114	1160	1134
DM	795	862	794	815	799	770	740	797	793	796
PW	995	1046	1040	979	1039	987	1028	983	1005	1030
MB	878	890	898	882	877	872	831	835	824	826
BB	914	866	851	901	861	791	802	864	815	886
DF	779	769	803	780	748	729	737	724	727	763
TS	916	930	963	988	989	971	910	941	969	1017
n	7	7	7	7	7	7	7	7	7	7
Mean	920	933	929	931	923	896	882	894	899	922
SEM	54	56	54	53	58	60	62	53	61	55

MEAN POWER OUTPUT (W)

A. 30 s Rest Interval Series

Subject	Sprint # 1	2	3	4	5	6	7	8	9	10
CrS Group										
Pre Supplementation										
MC	659	624	603	635	596	601	448.6	459	453	466
JM	622	697	609	719	642	632	515	587	557	604
LBC	551	606	624	642	615	624	528	543	555	557
NW	558	636	524	585	488	521	545	435	431	451
JS	772	709	743	751	714	692	657	564	546	501
DL	662	701	704	618	628	579	530	426	399	438
ML	632	664	634	613	624	600	572	480	525	510
n	7	7	7	7	7	7	7	7	7	7
Mean	637	662	634	652	615	607	542	499	495	504
SEM	28	16	27	23	26	20	24	25	25	23
Post Supplementation										
MC	783	792	776	761	770	711	586	591	575	588
JM	628	654	676	650	590	621	550	557	564	583
LBC	623	603	675	652	656	533	585	540	559	587
NW	558	587	560	535	467	576	554	505	488	488
JS	868	820	816	793	831	785	750	650	655	655
DL	655	651	619	612	691	661	625	531	564	586
ML	606	638	625	650	646	617	620	523	549	534
n	7	7	7	7	7	7	7	7	7	7
Mean	674	678	678	665	664	643	610	557	565	574
SEM	42	35	34	33	45	32	26	19	19	20
CON Group										
Pre Supplementation										
BS	876	821	767	736	711	652	621	495	529	506
DM	580	579	574	513	550	481	412	401	405	389
PW	716	727	721	682	722	701	564	549	564	549
MB	605	646	586	656	676	691	675	562	552	608
BB	592	552	576	559	595	579	528	511	464	542
DF	537	592	547	585	594	575	586	467	493	502
TS	607	602	617	593	609	558	550	493	494	538
n	7	7	7	7	7	7	7	7	7	7
Mean	645	646	627	618	637	605	562	497	500	519
SEM	44	36	32	29	25	30	31	20	21	25
Post Supplementation										
BS	845	871	776	746	677	648	619	493	539	535
DM	541	592	535	590	509	489	407	437	421	454
PW	736	691	654	703	669	682	527	587	605	595
MB	629	611	645	645	713	717	677	605	598	607
BB	617	642	646	651	645	626	627	596	490	525
DF	565	605	566	538	585	580	577	475	511	510
TS	613	586	613	545	613	600	615	491	551	519
n	7	7	7	7	7	7	7	7	7	7
Mean	649	657	634	631	630	620	578	526	531	535
SEM	40	38	29	30	26	28	34	26	24	20

MEAN POWER OUTPUT (W)

B. 6 min Rest Interval Series

Subject	Sprint # 1	2	3	4	5	6	7	8	9	10
CrS Group										
Pre Supplementation										
MC	718	730	711	760	753	726	736	698	709	743
JM	550	626	631	653	676	678	507	553	544	603
LBC	601	575	593	612	632	642	637	661	552	645
NW	559	557	579	585	596	626	556	466	522	562
JS	689	646	739	804	740	730	632	592	584	671
DL	698	748	689	643	712	681	583	690	674	724
ML	626	667	670	678	699	641	610	621	610	659
n	7	7	7	7	7	7	7	7	7	7
Mean	634	650	659	676	687	675	609	612	599	658
SEM	26	27	23	30	22	16	27	31	26	24
Post Supplementation										
MC	778	855	880	825	883	752	738	779	821	838
JM	646	699	693	676	718	621	640	655	662	665
LBC	643	684	686	710	712	615	623	635	616	704
NW	517	506	547	538	581	532	544	437	477	477
JS	785	884	831	813	824	818	675	606	602	519
DL	730	855	776	771	784	761	578	714	760	776
ML	631	729	655	687	750	761	671	625	665	678
n	7	7	7	7	7	7	7	7	7	7
Mean	676	745	724	717	750	694	638	636	658	665
SEM	36	50	43	37	36	30	25	40	42	49
CON Group										
Pre Supplementation										
BS	741	824	848	859	921	912	801	783	811	862
DM	438	456	476	449	455	369	362	452	428	430
PW	671	725	795	810	795	636	710	609	663	586
MB	605	627	648	633	696	689	559	566	599	614
BB	650	571	576	588	652	654	607	599	648	611
DF	508	509	564	590	607	602	485	532	551	561
TS	683	745	751	711	745	782	631	646	672	697
n	7	7	7	7	7	7	7	7	7	7
Mean	614	637	665	663	696	663	594	598	625	623
SEM	40	51	52	53	56	63	55	39	45	50
Post Supplementation										
BS	819	902	916	928	917	916	765	777	793	756
DM	572	600	521	577	576	567	515	506	498	504
PW	733	736	738	693	689	580	620	640	687	736
MB	589	625	664	653	636	643	621	602	578	567
BB	636	487	626	625	708	574	573	538	500	635
DF	536	568	615	600	537	509	552	546	560	585
TS	626	685	766	764	779	749	613	663	656	633
n	7	7	7	7	7	7	7	7	7	7
Mean	644	658	692	691	692	648	608	610	610	631
SEM	37	51	48	46	49	53	30	35	41	34

PLASMA [NH_3^+] ($\mu mol \cdot l^{-1}$)

A. 30 s Rest Interval Series

Subject	Samples						
	Rest	E5	E10	R2	R5	R10	R20
CrS Group							
			Pre Supplementation				
MC	25.8	140.4	224.4	241.5	227.0	230.4	166.7
JM	17.8	76.9	136.0	154.0	123.5	93.4	52.8
LBC	17.5	101.6	180.0	168.0	137.0	91.6	60.2
NW	23.0	94.1	151.0	207.9	200.1	192.3	132.2
JS	11.6	108.2	181.8	170.5	167.9	132.9	67.1
DL	47.1	106.5	231.3	238.9	225.0	172.0	134.6
ML	25.3	87.2	170.4	189.3	189.7	178.7	119.0
n	7	7	7	7	7	7	7
Mean	24.0	102.1	182.1	195.7	181.5	155.9	104.7
SEM	4.3	7.6	13.3	13.2	15.4	19.7	16.8
			Post Supplementation				
MC	20.1	115.6	233.0	244.5	227.8	213.4	166.9
JM	29.7	76.8	158.4	172.1	156.6	125.0	67.4
LBC	21.2	89.7	151.1	148.2	127.7	116.3	74.7
NW	17.7	21.0	73.2	88.6	123.9	38.9	13.4
JS	9.1	80.9	185.6	211.8	169.1	142.2	87.0
DL	46.7	57.5	111.3	215.9	227.7	193.6	116.5
ML	6.7	92.9	115.9	131.5	143.4	132.6	114.4
n	7	7	7	7	7	7	7
Mean	21.6	76.3	146.9	173.2	168.0	137.4	91.5
SEM	5.1	11.4	20.0	20.7	16.5	21.4	18.1
CON Group							
			Pre Supplementation				
BS	17.0	166.9	277.8	282.6	281.7	252.4	176.1
DM	9.8	30.5	85.2	126.5	115.1	93.4	52.1
PW	29.0	114.4	205.3	221.6	213.4	185.4	111.2
MB	38.3	84.2	124.4	169.6	167.9	154.9	102.1
BB	28.8	70.4	105.3	99.5	107.1	66.5	28.9
DF	25.2	49.6	110.3	118.9	128.7	70.6	38.4
TS	9.4	87.3	171.3	187.7	188.2	177.2	103.1
n	7	7	7	7	7	7	7
Mean	22.5	86.2	154.2	172.3	171.7	142.9	87.4
SEM	4.1	16.9	25.9	24.4	23.6	26.1	19.5
			Post Supplementation				
BS	14.0	195.5	281.2	277.9	274.7	284.4	177.1
DM	22.1	44.0	111.1	116.1	150.5	119.9	74.3
PW	17.6	108.6	179.0	135.7	135.7	145.4	110.1
MB	36.2	81.5	151.0	160.0	171.6	149.8	88.9
BB	27.7	58.1	123.6	120.1	116.1	77.0	26.8
DF	33.6	46.1	93.1	158.6	151	109.2	56.8
TS	11.5	213	89.1	240.7	234.4	217	139.6
n	7	7	7	7	7	7	7
Mean	23.2	106.7	146.9	172.7	176.3	157.5	96.2
SEM	3.6	26.6	25.4	23.6	21.6	26.8	19.2

PLASMA [NH_3^+] ($\mu mol \cdot l^{-1}$)

B. 6 min Rest Interval Series

Subject	Samples						
	Rest	E5	E10	R2	R5	R10	R20
CrS Group							
			Pre Supplementation				
MC	38.5	54.2	34.1	43.4	38.3	18.0	16.6
JM	23.8	29.2	36.9	43.8	11.8	14.8	17.5
LBC	28.8	49.8	50.7	47.2	48.0	45.2	21.5
NW	30.6	38.1	24.3	26.4	21.8	19.0	16.7
JS	15.1	32.6	20.8	17.2	16.2	17.3	16.9
DL	38.0	46.8	39.1	38.1	31.5	35.4	43.5
ML	14.8	29.3	23.8	18.2	23.5	12.4	12.1
n	7	7	7	7	7	7	7
Mean	27.1	40.0	32.8	33.5	27.3	23.2	20.7
SEM	3.7	3.9	4.0	4.8	4.8	4.6	3.9
			Post Supplementation				
MC	18.8	26.1	25.5	27.5	21.1	5.5	8.6
JM	17.9	17.3	19.2	14.5	9.8	7.4	5.0
LBC	18.7	10.5	14.9	18.7	12.8	11.5	10.9
NW	21.2	10.4	5.3	5.1	3.7	9.9	20.2
JS	14.3	12.0	16.3	5.8	6.5	18.8	17.8
DL	22.8	22.2	18.3	14.5	14.3	15.6	12.0
ML	20.6	32.3	27.0	29.1	22.1	22.7	18.3
n	7	7	7	7	7	7	7
Mean	19.2	18.7	18.1	16.5	12.9	13.1	13.3
SEM	1.0	3.2	2.7	3.6	2.6	2.4	2.1
CON Group							
			Pre Supplementation				
BS	9.7	122.7	109.2	98.5	79.5	68.0	37.6
DM	27.1	33.1	25.0	19.7	23.7	10.8	4.6
PW	14.2	42.4	39.0	11.6	12.6	14.8	14.4
MB	15.7	14.6	12.9	19.5	11.5	12.1	10.6
BB	28.4	40.2	38.4	40.8	43.4	38.3	35.6
DF	25.8	28.2	19.0	17.5	17.7	14.3	17.3
TS	9.2	39.1	28.1	14.5	11.0	6.9	4.0
n	7	7	7	7	7	7	7
Mean	8.3	35.2	32.5	30.9	25.2	22.1	13.8
SEM	3.1	13.3	12.3	11.7	9.5	8.3	5.2
			Post Supplementation				
BS	8.3	134.6	96.6	80.7	67.4	45.9	18.5
DM	26.6	29.5	29.1	27.7	27.7	22.9	23.9
PW	22.9	12.2	21.7	19.8	19.2	12.5	17.7
MB	8.3	21.9	8.2	5.6	5.5	5.7	3.4
BB	38.5	36.5	35.6	15.0	24.1	19.5	14.3
DF	21.6	13.1	4.3	5.6	12.7	10.4	21.8
TS	26.0	38.5	19.9	17.2	17.3	7.4	9.8
n	7	7	7	7	7	7	7
Mean	21.7	40.9	30.8	24.5	24.8	17.8	15.6
SEM	4.0	16.1	11.7	9.8	7.6	5.2	2.7

PLASMA [La^-] ($mmol \cdot l^{-1}$)

A. 30 s Rest Interval Series

	Samples						
Subject	Rest	E5	E10	R2	R5	R10	R20
CrS Group							
			Pre Supplementation				
MC	1.0	12.3	17.6	19.6	20.5	20.2	16.4
JM	1.1	10.8	15.3	15.8	15.0	13.0	9.4
LBC	1.5	10.1	16.3	17.4	16.1	13.6	9.8
NW	1.5	8.7	12.1	14.9	14.5	13.9	10.4
JS	1.3	9.8	13.1	12.7	12.5	10.5	5.3
DL	1.1	11.2	16.8	17.4	17.1	13.7	11.3
ML	1.7	11.4	17.0	18.8	18.6	18.0	13.6
n	7	7	7	7	7	7	7
Mean	1.3	10.6	15.5	16.6	16.3	14.7	10.9
SEM	0.1	0.4	0.8	0.9	1.0	1.2	1.3
			Post Supplementation				
MC	0.9	11.0	18.5	19.4	20.1	19.7	14.5
JM	1.2	7.2	11.8	18.3	17.9	16.4	12.3
LBC	1.3	9.1	14.8	16.0	15.6	15.3	13.3
NW	1.9	5.9	9.9	11.2	10.4	8.5	4.8
JS	1.3	10.8	15.3	16.6	16.8	15.1	10.4
DL	1.2	8.1	12.9	16.7	16.7	14.9	10.5
ML	1.6	13.7	16.7	17.9	18.1	17.2	12.8
n	7	7	7	7	7	7	7
Mean	1.3	9.4	14.3	16.6	16.5	15.3	11.2
SEM	0.1	1.0	1.1	1.0	1.2	1.3	1.2
CON Group							
			Pre Supplementation				
BS	1.5	16.9	21.7	23.1	23.6	22.5	16.6
DM	1.2	8.3	11.1	13.5	14.1	11.5	7.4
PW	1.4	10.2	16.1	18.1	18.1	17.1	11.8
MB	1.6	8.3	14.2	16.2	16.9	15.3	10.7
BB	1.1	7.8	11.2	12.8	13.6	12.3	8.0
DF	1.3	5.9	9.9	10.3	10.1	8.1	5.1
TS	1.1	9.1	14.5	15.7	16.3	15.5	11.0
n	7	7	7	7	7	7	7
Mean	1.3	9.5	14.1	15.7	16.1	14.6	10.1
SEM	0.1	1.3	1.5	1.6	1.6	1.7	1.4
			Post Supplementation				
BS	1.5	17.4	22.9	23.7	24.6	23.4	17.9
DM	1.2	8.2	11.5	13.8	14.0	11.9	7.5
PW	1.1	12.2	16.8	15.0	16.9	15.3	11.9
MB	1.9	7.1	10.3	10.3	9.1	6.7	3.4
BB	1.4	8.4	12.6	13.8	14.2	12.0	8.1
DF	1.3	6.9	10.7	13.5	12.7	9.9	6.2
TS	2.7	16.7	10.0	18.5	19.1	18.0	12.6
n	7	7	7	7	7	7	7
Mean	1.6	11.0	13.5	15.5	15.8	13.9	9.7
SEM	0.2	1.7	1.8	1.6	1.9	2.1	1.8

PLASMA [La^-] ($mmol \cdot l^{-1}$)

B. 6 min Rest Interval Series

Subject	Samples						
	Rest	E5	E10	R2	R5	R10	R20
CrS Group							
			Pre Supplementation				
MC	1.5	7.5	7.9	9.1	8.2	5.9	3.7
JM	1.3	9.5	8.3	8.3	7.2	5.7	4.0
LBC	1.4	4.4	5.3	6.2	5.1	4.1	2.8
NW	1.8	4.0	3.0	6.6	2.7	2.1	1.6
JS	1.3	6.3	3.6	3.8	3.0	2.4	1.8
DL	1.3	5.2	4.8	5.8	5.2	3.5	2.2
ML	1.6	7.2	6.6	6.4	7.3	5.3	3.2
n	7	7	7	7	7	7	7
Mean	1.5	6.3	5.7	6.6	5.5	4.2	2.8
SEM	0.1	0.7	0.8	0.7	0.8	0.6	0.3
			Post Supplementation				
MC	1.7	9.8	11.5	12.3	10.9	9.2	6.1
JM	1.2	6.9	9.5	9.6	8.6	7.0	4.9
LBC	1.4	5.6	6.2	6.9	6.3	4.9	3.5
NW	1.4	2.9	2.6	2.7	2.4	2.0	1.6
JS	1.7	6.9	4.6	5.1	4.3	3.3	2.3
DL	2.4	6.1	7.7	7.1	6.3	4.7	3.2
ML	1.7	7.2	7.8	8.2	7.2	5.5	3.6
n	7	7	7	7	7	7	7
Mean	1.6	6.5	7.1	7.4	6.6	5.2	3.6
SEM	0.1	0.8	1.1	1.2	1.1	0.9	0.6
CON Group							
			Pre Supplementation				
BS	1.5	13.4	13.8	14.7	13.8	10.8	6.3
DM	1.1	5.3	6.4	6.3	5.4	4.1	2.7
PW	1.2	6.4	6.5	6.7	5.8	4.4	3.0
MB	1.5	5.3	4.6	4.1	4.4	3.5	2.0
BB	2.6	4.8	6.1	6.9	5.4	4.2	1.6
DF	1.1	5.2	3.7	4.3	4.0	2.8	2.0
TS	1.9	7.6	7.2	7.4	6.3	5.0	3.1
n	7	7	7	7	7	7	7
Mean	1.6	6.8	6.9	7.2	6.5	5.0	3.0
SEM	0.2	1.1	1.2	1.3	1.3	1.0	0.6
			Post Supplementation				
BS	1.9	13.8	12.8	13.0	11.3	8.6	5.3
DM	1.4	5.0	5.8	6.7	5.8	4.4	2.8
PW	1.4	4.1	4.6	5.7	4.8	3.6	2.5
MB	1.7	3.2	2.7	2.7	2.0	2.2	2.5
BB	1.4	4.6	3.0	3.6	3.2	2.4	1.8
DF	1.5	4.0	3.3	4.3	3.6	2.8	2.7
TS	1.3	6.4	4.4	4.9	4.5	3.1	2.1
n	7	7	7	7	7	7	7
Mean	1.5	5.9	5.2	5.8	5.0	3.9	2.8
SEM	0.1	1.4	1.3	1.3	1.1	0.8	0.4

PLASMA [H^+] ($nmol \cdot l^{-1}$)

A. 30 s Rest Interval Series

Subject	Samples						
	Rest	E5	E10	R2	R5	R10	R20
CrS Group							
			Pre Supplementation				
MC	37.8	53.3	56.3	62.2	66.8	72.8	59.7
JM	37.7	54.9	60.3	63.1	56.6	50.8	43.1
LBC	41.5	51.5	64.0	64.6	62.2	59.8	51.3
NW	37.8	52.3	58.7	65.5	63.0	57.3	46.3
JS	39.0	55.7	62.3	63.3	57.3	51.2	42.1
DL	39.1	59.5	73.3	69.2	67.1	61.0	49.7
ML	38.2	56.6	66.8	68.0	70.6	66.2	55.1
n	7	7	7	7	7	7	7
Mean	38.7	54.8	63.1	65.1	63.3	59.9	49.6
SEM	0.5	1.0	2.1	1.0	2.0	3.0	2.4
			Post Supplementation				
MC	36.8	52.2	59.0	66.1	71.2	70.1	59.0
JM	37.5	58.3	71.9	66.1	63.7	55.4	44.8
LBC	37.3	51.7	62.5	63.8	64.8	59.9	51.2
NW	40.2	51.5	54.5	53.3	50.8	45.9	40.6
JS	36.0	53.1	66.0	68.0	61.7	56.3	46.7
DL	39.9	56.4	70.5	65.2	66.9	60.0	49.9
ML	37.2	60.4	73.1	66.6	68.3	65.5	53.1
n	7	7	7	7	7	7	7
Mean	37.8	54.8	65.4	64.2	63.9	59.0	49.3
SEM	0.6	1.3	2.7	1.9	2.5	2.9	2.3
CON Group							
			Pre Supplementation				
BS	38.8	54.9	61.1	63.8	65.1	58.5	48.6
DM	36.2	54.1	67.2	69.1	66.3	61.8	49.1
PW	37.9	67.3	80.0	80.0	80.9	73.8	55.5
MB	39.4	51.3	53.4	51.7	49.2	48.2	42.5
BB	37.0	48.8	54.2	53.9	51.5	48.9	42.1
DF	39.1	46.2	55.3	52.8	50.9	46.6	42.5
TS	37.1	57.6	66.8	66.0	65.0	61.2	49.9
n	7	7	7	7	7	7	7
Mean	37.9	54.3	62.6	62.5	61.3	57	47.2
SEM	0.5	2.6	3.6	3.9	4.3	3.7	1.9
			Post Supplementation				
BS	39.4	55.5	60.7	62.7	62.9	56.4	48.8
DM	37.7	60.7	68.3	70.3	68.5	63.2	50.4
PW	37.5	66.1	87.7	84.5	81.3	80.7	60.7
MB	38.6	47.6	53.6	50.7	47.4	43.5	39.2
BB	38.2	50.8	55.3	56.2	54.6	49.8	44.2
DF	39.1	55.5	60.5	57.2	54.8	50.6	44.0
TS	41.9	61.5	72.0	72.9	67.9	67.0	54.6
n	7	7	7	7	7	7	7
Mean	38.9	56.8	65.5	64.9	62.5	58.7	48.8
SEM	0.6	2.4	4.5	4.4	4.3	4.8	2.7

PLASMA [H^+] ($nmol \cdot l^{-1}$)

B. 6 min Rest Interval Series

Subject	Samples						
	Rest	E5	E10	R2	R5	R10	R20
CrS Group							
			Pre Supplementation				
MC	38.8	44.1	56.3	46.1	46.6	43.2	43.2
JM	38.6	43.5	41.4	42.8	41.4	40.2	40.2
LBC	39.3	43.7	44.8	46.0	44.4	42.6	48.2
NW	39.4	43.5	42.5	44.6	43.4	41.5	39.4
JS	36.8	41.9	38.4	40.5	39.5	40.0	38.5
DL	39.7	43.8	42.8	46.8	44.3	41.1	40.1
ML	36.9	44.9	43.1	45.0	42.7	42.3	39.4
n	7	7	7	7	7	7	7
Mean	38.5	43.6	44.2	44.5	43.2	41.6	41.3
SEM	0.4	0.3	2.2	0.8	0.9	0.4	1.3
			Post Supplementation				
MC	37.7	41.2	56.2	46.1	46.6	44.3	40.9
JM	37.3	42.0	41.4	42.8	41.4	40.2	38.9
LBC	36.6	42.9	44.8	46.0	44.4	48.2	39.0
NW	39.4	43.5	42.5	44.6	43.4	41.5	39.4
JS	36.8	41.9	38.4	40.5	39.5	40.0	38.5
DL	39.7	43.8	42.8	46.8	44.3	41.1	40.1
ML	36.9	44.9	43.1	45.0	42.7	42.3	39.4
n	7	7	7	7	7	7	7
Mean	37.8	42.9	44.2	44.5	43.2	42.5	39.5
SEM	0.5	0.5	2.1	0.8	0.9	1.1	0.3
CON Group							
			Pre Supplementation				
BS	38.6	45.8	46.4	45.1	45.0	41.8	41.8
DM	39.7	45.4	44.0	46.4	43.9	41.7	41.7
PW	37.8	45.0	45.6	50.9	47.3	42.2	38.9
MB	39.1	40.2	40.3	39.4	39.3	37.3	38.2
BB	37.7	40.4	40.3	42.8	41.2	40.1	38.9
DF	38.6	45.0	43.8	46.0	43.7	41.6	39.9
TS	38.5	43.7	43.3	46.2	43.6	41.1	39.8
n	7	7	7	7	7	7	7
Mean	38.6	43.7	43.4	45.3	43.4	40.8	39.9
SEM	0.3	0.9	0.9	1.3	1.0	0.6	0.5
			Post Supplementation				
BS	40.2	45.1	46.3	45.1	45.0	41.8	40.6
DM	38.7	42.7	44.0	46.3	43.9	41.7	40.2
PW	38.2	47.2	44.1	47.5	45.1	40.6	37.5
MB	41.8	43.3	41.9	41.8	41.3	40.6	41.0
BB	37.0	38.3	38.3	40.5	39.3	38.1	37.6
DF	41.5	44.5	43.3	44.8	42.9	41.5	41.2
TS	39.4	45.6	40.5	43.5	42.9	41.6	38.9
n	7	7	7	7	7	7	7
Mean	39.5	43.8	42.6	44.2	42.9	40.8	39.6
SEM	0.7	1.1	1.0	0.9	0.8	0.5	0.6