THE EFFECTS OF PRIOR ORAL CREATINE SUPPLEMENTATION ON PERFORMANCE AND METABOLISM AFTER 7 DAYS OF SPRINT CYCLE TRAINING

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Declaration

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CAPE TOWN

Abstract

Oral creatine supplementation has been shown to increase skeletal muscle total creatine (TCr) content, and in some cases improve performance in high-intensity short duration exercise. A variety of factors related to an enhanced efficacy of adenine nucleotide metabolism have been demonstrated as partly responsible for this ergogenic effect. Also, there is evidence that high-intensity sprint training results in a decrease in muscle total adenine nucleotide (TAN) and/or ATP stores. This placebo controlled double-blind study examined whether an oral creatine supplementation regimen would 1) increase muscle TCr content, 2) attenuate any loss in TAN or ATP during intermittent sprint training, and 3) have an ergogenic effect on performance after sprint training. Thirteen male endurance trained cyclists ingested 20 g of creatine monohydrate supplement or placebo per day for 7 days, after which they ingested a maintenance dose of 2 g creatine or placebo per day for the remainder of the trial (15d). While on the maintenance dose, subjects performed intermittent sprint training (ST) on a cycle ergometer (10 x 10 s sprints with 140 s active recovery) for 6 consecutive days and a 7th day after one day of rest. Performance tests were performed before and after ST, and metabolic tests were performed on the 1st and 7th day of ST. TCr increased significantly with creatine supplementation (creatine group pre: 121 ± 4, post: 147 ± 9; vs. placebo group pre: 122 ± 4, post: 125 ± 4 mmol/kg dm; mean ± SEM; $p<0.05$). The increase in TCr correlated with the percentage Type IIB fibres ($r=0.95$, $p<0.005$). By day 7 of ST, TCr content was no longer significantly higher than pre-supplementation levels despite the maintenance dose of creatine. ST resulted in a significant decrease in resting muscle TAN and ATP content in both groups (ATP content in creatine group pre: 24.1 ± 0.8, post: 17.2 ± 0.5; and placebo group pre: 26.5 ± 1.1, post: 18.0 ± 0.6 mmol/kg dm; $p<0.001$). During and in recovery from ST on day 7, both groups had lower plasma ammonia ($p<0.05$), hypoxanthine ($p<0.001$) and urate ($p<0.001$) accumulation than on day 1 of ST. There was no improvement in 1-hr cycle distance performance after ST, but peak sustained power output increased in the creatine group and not in the placebo group after ST ($p<0.05$). Peak and mean power during a 30 s Wingate test increased significantly ($p<0.05$) after ST but there was no additional ergogenic effect of creatine supplementation. In conclusion, this study shows that 1) the efficacy of muscle creatine uptake was dependent on the percentage of Type IIB fibres, 2) creatine supplementation and maintenance (2 g/d) did not attenutate ATP or TAN loss during 7 days of ST, 3) ST decreased the accumulation of plasma products of adenine nucleotide degradation and improved 30 s sprint performance, and 4) creatine supplementation and ST did not improve 1-hr cycle distance performance.

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List of Abbreviations

t	time
WL	workload
UV	ultraviolet
HPLC	high performance liquid chromatography
PSPO	peak sustained power output
W	watts
Pi	inorganic phosphate
ATP	adenosine 5' triphosphate
ADP	adenosine 5' diphosphate
AMP	adenosine 5' monophosphate
TAN	total adenine nucleotide
IMP	inosine monophosphate
PCr	phosphocreatine
Cr	creatine
TCr	total creatine
Cn	creatinine
Hx	hypoxanthine
NH_3	ammonia
NAD	nicotinamide adenosine dinucleotide
WAT	Wingate anaerobic power test
AN	adenine nucleotide
PNC	purine nucleotide cycle
rpm	revolutions per minute
ST	sprint training
CV	coefficient of variation
dm	dry mass
NMR	nuclear magnetic resonance
MAOD	maximal accumulated oxygen deficit
PFK	phosphofructokinase
LDH	lactate dehydrogenase
HPRT	hypoxanthine phosphoribosyl transferase
MK	myokinase
AK	adenylate kinase
CPK	creatine phosphokinase
PCA	perchloric acid
K_2CO_3	potassium carbonate
KH_2PO_4	potassium dihydrogen orthophosphate
TBAHS	tetrabutylammonium hydrogen sulphate
KOH	potassium hydroxide
ANOVA	analysis of variance
NS	not significant
SEM	standard error of the mean

CHAPTER 1:

INTRODUCTION

Ergogenic aids may maximise the training and preparation for an athletic event, or improve performance during the event itself. The study of nutritional aids to performance has been of interest to both the scientist and the athlete for many years. Such studies often stem from other studies that examined factors which limit performance, and/or contribute to muscle fatigue.

One of these observations is that the rapid depletion of intramuscular creatine phosphate (PCr) stores during high intensity exercise is associated with muscular fatigue (Sahlin and Ren, 1989). Despite no direct effect of PCr and creatine on actomyosin function, there appears to be a linear relationship between the onset of muscular fatigue and the decreasing levels of PCr. Hence the maintenance of high PCr stores and a high pre-exercise level of PCr would be optimal in delaying the onset of fatigue and maximising total force production during an exercise bout/s (Hultman et al., 1991; Hultman et al., 1967).

Recently it has been shown that oral creatine supplementation can increase muscle creatine and PCr stores significantly (Harris et al., 1992; Greenhaff et al., 1994a; Greenhaff et al., 1994A). This "creatine loading" phenomenon is analogous to the concept of increasing muscle glycogen content by carbohydrate ingestion. Research studies on this topic are relatively contemporary, and consequently in this thesis reference will be made to both published articles and to abstracts for which no full-length publication is yet available (the latter will be represented by an "A" following the year of publication).

In addition to increasing muscle total creatine (TCr) content, oral creatine supplementation has been shown to enhance high-intensity short duration exercise performance (Balsom et al., 1993; Birch et al., 1994; Greenhaff et al., 1993; Hall et al., 1995A; Jacobs et al., 1995A; Lemon et al., 1995A; Viru et al., 1993). In contrast, other studies have not shown improved performance with creatine supplementation (Almada et al., 1995A; Burke et al., 1995A; Cooke et al., 1995; Grindstaff et al., 1995A; Redondo et al., 1995A).

This may be due to insufficient uptake of creatine into the muscle, since large variations in individual changes in creatine stores in response to supplementation have been shown (Greenhaff et al., 1994a; Harris et al., 1992; Lemon et al., 1995A). The factors which influence the extent of creatine uptake need to be elucidated, but may include relatively low baseline (pre-supplementation) levels of total creatine (Greenhaff et al., 1994a; Harris et al., 1992).

The exact mechanism for an ergogenic effect is not yet clear, but from the alteration in metabolic effects of exercise that have been reported following creatine supplementation, it has been suggested that creatine loading has a beneficial effect on adenine nucleotide (AN) metabolism. Proposed mechanisms through which this occurs during exercise include increased PCr availability (Harris et al., 1992) and more rapid PCr resynthesis (Greenhaff et al., 1994a), better maintenance of adenosine 5'-triphosphate (ATP) concentrations, and perhaps a reduction in the extent of ATP loss during the later bouts of repeated high-intensity exercise (Greenhaff et al., 1994A).

Maximal high-intensity (sprint) exercise is characterised by an acute decrease in muscle total adenine nucleotide (TAN) and ATP content (Cheetham et al., 1986; Gaitanos et al., 1993; Sahlin and Broberg, 1990; Tullson et al., 1995), and similarly there is evidence that high-intensity *training* results in a depression of TAN or ATP stores which is not recovered immediately (Green et al., 1987; Hellsten-Westing et al., 1993b; Stathis et al., 1994). Therefore, any factors which may prevent or attenuate the loss of AN from the muscle pool warrant examination. As discussed above, it is evident that an increase muscle TCr content affects the AN pathway during high-intensity exercise. It is hypothesised that creatine supplementation before and during high-intensity exercise training may attenuate the decrease in AN stores, and that this effect may manifest as an improvement in performance during and after training.

In summary, the specific aims of this study are:

1) to examine whether skeletal muscle fibre type composition influences the efficacy of creatine loading in human skeletal muscle;

2) to examine whether a loss in adenine nucleotide content as a result of consecutive days of intermittent sprint training is attenuated by prior creatine loading; and,

3) to assess the effect of one week of concentrated intermittent sprint training on both sprint and high-intensity endurance performance, with and without prior creatine loading.

CHAPTER 2:

LITERATURE REVIEW

2.1 Creatine: structure and origin

Creatine (Cr) is a component of human skeletal muscle that can be derived from the diet or it can be endogenously synthesised. Approximately 95% of the body's creatine pool is in the skeletal muscle, and the other 5% is predominantly in the heart, brain and testes (Balsom et al., 1994). Consequently, endogenously synthesised creatine must be transported in the blood to the muscle.

At rest, creatine occurs in the muscle in two forms: free creatine (Cr, 35%) and phosphocreatine (PCr, 65%). The sum of these is the muscle TCr. Analysis of muscle biopsies from healthy individuals shows typical TCr contents between 120 and 130 mmol/kg dry mass (dm)(which is approximately 120 g in a 70 kg male) (Harris et al., 1974; Harris et al., 1992). The phosphocreatine content of Type II muscle fibres has been shown to be between 10% and 13% higher than that in Type I fibres when measured in single muscle fibres (Greenhaff et al., 1994b; Tesch et al., 1989 respectively); or up to 25% higher when comparing the levels in human vastus lateralis versus the predominantly Type I soleus muscle (Edstrom et al., 1982).

Creatine and PCr are broken down nonenzymatically to creatinine which is not reconvertible to creatine in significant amounts (Bessman and Carpenter, 1985). Creatinine excretion does not necessarily match creatine synthesis, but rather is excreted at a constant fraction of the creatine body pool, that is 1.6% of the total creatine pool per day in humans (Crim et al., 1976). Thus, in a 70 kg male with a TCr content of approximately 120 g, the turnover rate of creatine would be approximately 2 g per day. This is replaced by both endogenous and exogenous sources.

Biosynthesis of creatine from the amino acids arginine, glycine and methionine occurs in the liver, pancreas and kidney. It appears that endogenous synthesis is regulated in part by dietary intake (exogenous sources) via feedback mechanisms. Exogenous sources of creatine include foodstuffs such as raw meat and fish which have a high creatine content.

Creatine is found in some plant products in trace amounts only, hence daily intake of creatine is limited in a vegetarian or vegan diet (Delanghe et al., 1989), and daily requirements of creatine rely on its endogenous synthesis (Balsom et al., 1994). A study including two vegetarian subjects reported that their total muscle creatine contents were low, but within the normal range (Harris et al., 1992). The noticeably lower *serum* creatine levels observed in vegetarians previously (Delanghe et al., 1989), was accompanied by lower urinary creatinine excretion, and this was most probably a result of a mechanism to maintain muscle creatine content.

The slow efflux of creatine from muscle tissue prevents large fluctations in muscle creatine content in both rest and exercise. Consequently, the body creatine pool has been viewed as remarkably stable. However, it has been shown that the administration of precursor amino acids arginine and glycine can increase the rate of creatine synthesis (Crim et al., 1976), and more recently that the size of the TCr pool can be manipulated by ingestion of large quantities of creatine (Harris et al., 1992).

2.2 Creatine: functions related to exercise

It is well accepted that phosphocreatine, free creatine and inorganic phosphate (P_i) play pivotal roles in the regulation and homeostasis of skeletal muscle energy metabolism. In addition, the maintenance of PCr content is important for continued force production during high-intensity exercise (Greenhaff et al., 1994a; Bessman and Carpenter, 1985). Indeed, the decline in force production during sustained static contractions and the restoration of force during the recovery period have been shown to be closely related to PCr levels (Sahlin and Ren, 1989).

During exercise, the hydrolysis of ATP to adenosine-5'-diphosphate (ADP) and P_i releases energy required to fuel muscle contraction. Due to the relatively small amount of ATP that is stored in the muscle (4 mM) (Guyton, 1986), it must be continually resynthesised from secondary energy sources. These sources include immediate resynthesis from PCr;

the pathways of glycolysis and glycogenolysis; and the more sustained process of oxidative phosphorylation in the muscle mitochondria.

Immediate resynthesis of hydrolysed ATP from its product ADP occurs by the provision of a high-energy phosphate, P_i, which is released from the breakdown of PCr to creatine. This allows the regeneration of ATP at, or near to, the active site of ATPases at the myofibril. The rate of this reaction is stimulated by relatively small declines in ATP concentration and the rise in concentrations of ADP and protons associated with muscular contraction (Sahlin and Broberg, 1990). During exercise of high-intensity and short duration, PCr levels may decrease to as low as 10-20% of the resting pre-exercise level (Greenhaff et al., 1994a; Harris et al., 1976). Although PCr degradation occurs in both skeletal muscle fibre types during high-intensity exercise, the extent and rate of degradation has been shown to be markedly greater in Type II than Type I fibres during a 30 s treadmill sprint (Greenhaff et al., 1994b) and 30 maximal knee extensions (Tesch et al., 1989).

The result of PCr degradation is a rise in the concentration of creatine close to the myofibrils. In order to maintain PCr levels, creatine is rephosphorylated at the mitochondria in the presence of oxygen. This process will be described in more detail later. The resynthesis of PCr is rapid and includes a fast and a slow component. After dynamic exercise approximately 84% of the deficit in PCr is restored within two mins of the cessation of exercise, and complete recovery is evident after 20 mins (Harris et al., 1976). The recovery in the total amount of PCr has been shown to be significantly lower after isometric compared to dynamic exercise (Harris et al., 1976). This is probably due to comparatively reduced skeletal muscle blood flow (and hence aerobic potential) characteristic of the former type of exercise. Also, Tesch et al. (1989) reported that the initial rate of PCr resynthesis is greater in Type I compared to Type II muscle fibres, which again, is in accordance with the greater aerobic capacity of Type I fibres.

Since PCr can be used to near depletion easily, the amount of PCr stored in the muscle is an important factor when considering the extent to which it can contribute as a secondary energy source - especially during maximal high-intensity exercise. A reduction in the resting levels of PCr will result in a greater reliance on other secondary sources of ATP resynthesis such as glycolysis and glycogenolysis. During high-intensity exercise, oxygen-independent glycolysis results in lactate and proton accumulation. The latter has been shown to be linked to poor acto-myosin cross-bridge function (albeit indirectly via a decrease in pH) and decreased muscular force production (Sahlin, 1992). Thus, during high-intensity exercise it is preferable to delay the reliance on oxygen-independent glycolytic ATP resynthesis. The rapid provision of energy by PCr and its efficient resynthesis makes it the major and preferred substrate for the resynthesis of ATP in muscle at the onset of exercise or during single or multiple bouts of exercise of high-intensity and short duration.

Another biochemical function of creatine is that of an energy carrier between the mitochondria and the cytosolic utilisation sites. This is integrally related to the need to rephosphorylate creatine after PCr breakdown (as discussed previously), and the secondary resynthesis of ATP by mitochondrial oxidative phosphorylation. Creatine accumulation (from PCr breakdown) at the myofibril makes its diffusion gradient towards the mitochondria high. Thus creatine diffuses to the mitochondrial membrane where creatine kinase (bound outside the inner mitochondrial membrane) catalyses the resynthesis of PCr at the expense of mitochondrial ATP. The ADP formed stimulates ATP resynthesis by oxidative phosphorylation in the mitochondria. Although a small amount of ATP diffuses from the mitochondria to utilisation sites, it is predominantly creatine that transports the high-energy phosphate in the form of PCr back to the myofibril. Thus PCr functions as an energy carrier between the mitochondria and cytosolic utilisation sites. This mechanism is known as the "creatine-phosphocreatine shuttle", and it is based on the view that adenine nucleotides and creatine kinases are compartmentalised (Bessman and Carpenter, 1985).

There is, however, evidence that does not favour the importance of the shuttle mechanism in steady state intracellular diffusion of high-energy phosphate between mitochondria and myofibrils (Meyer, 1989). In a study using the creatine analogue β-guanidinopropionic acid, Meyer (1989) showed that the normal creatine content of skeletal muscle is far in excess of what is required to support the shuttle effect. This result is consistent with a linear model of respiratory control by cytoplasmic nucleotides, in which the creatine-kinase system acts as an adenylate buffer or chemical capacitor rather than an energy carrier.

During submaximal exercise, the major contributors to energy requirements are the oxidative glycolytic-glycogenolytic systems and oxidative phosphorylation - the latter contributing between 40% and 70% depending on the workload (for a review see Hawley and Hopkins, 1995). Although PCr is not required to provide P_i for ADP rephosphorylation immediately and rapidly during such exercise, it is through the shuttle mechanism that it plays a key regulating role. A possible benefit of increasing muscle creatine stores may be an improved capacity to transport energy via the shuttle, thus increasing the contribution of oxidative processes to the energy requirements and decreasing the utilisation of oxygen-independent glycolysis and glycogenolysis. In addition, related to the inhibition of glycolysis by high concentrations of PCr, both creatine and P_i aid the regulation of key glycolytic enzyme activities (such as phosphofructokinase (PFK), glyceraldehyde 3-phosphate dehydrogenase, and pyruvate kinase) and have a role in pH buffering (Walker, 1979).

In addition to its role in energy transport and energy supply for muscle contraction, phosphocreatine also provides energy for protein synthesis. At least 70% of protein synthesis is dependent on the phosphagen energy source. Due to increased cycling of the creatine-phosphocreatine shuttle during exercise, the delivery of PCr to protein synthesising sites is increased, and thus may influence the muscle hypertrophy associated with exercise (Bessman and Carpenter, 1985).

2.3 Adenine nucleotide metabolism

From the discussion above, it is clear that creatine is integrally related to adenine nucleotide (AN) metabolism. The regulation of AN metabolism is of particular importance during exercise when large, abrupt increases in the rate of energy transfer occur and thus the efficient control of nucleotide concentrations and their interconversions is essential.

2.3.1 Metabolic pathways

When AN degradation occurs, the concentrations of degradation products increase intramuscularly. Some of these may be used in the resynthesis of AN in the myocyte, while other compounds diffuse out of the muscle cell into the blood and can be described as being "lost" AN.

During exercise, the energy demand of force production necessitates the hydrolysis of ATP by myosin ATPase. When the rate of ATP hydrolysis exceeds the rate of resynthesis, the concentrations of free ADP and adenosine 5'-monophosphate (AMP) increase. During maximal high-intensity exercise there is a decreased capacity to rephosphorylate ADP for the resynthesis of ATP. In order to maintain a high ATP:ADP ratio at the myofibril (essential for the continuation of contraction), it is necessary that the myokinase reaction (ADP + ADP <=> ATP + AMP) favours ATP production (for review see Tullson and Terjung, 1991). To facilitate this, and stabilise the relative ratios of ATP to ADP and AMP in this equilibrium, the further breakdown of the AMP that has accumulated from ATP hydrolysis is required. AMP can be broken down via two pathways. Firstly, it can be dephosphorylated to adenosine; and secondly, it can be deaminated by AMP deaminase to form inosine monophosphate (IMP), and ammonia (NH_3) is released. The latter pathway is the main route of degradation in skeletal muscle (Sahlin et al., 1978).

Ammonia produced by AMP deamination diffuses readily from the muscle into the blood (for review see Banister and Cameron, 1990) and during high-intensity exercise the concentration of this compound in the plasma provides a valid indication of intramuscular AN degradation. The extent of IMP and NH_3 accumulation is dependent on the severity of the metabolic stress or energetic imbalance (which is determined by the intensity and duration of exercise) (Sahlin and Broberg, 1990). Acute decreases in intramuscular ATP concentration can be stochiometrically matched with IMP accumulation (Meyer et al., 1980; Sahlin et al., 1978; Sahlin and Broberg, 1990). This can amount to 15% (Sahlin et al., 1978; Sahlin and Broberg, 1990), 20% (Tullson et al., 1995), or even 32% (Gaitanos et al., 1993) of the pre-exercise muscle ATP content when exercising maximally.

When the energy demand persists, IMP is further degraded to inosine, hypoxanthine, xanthine and finally uric acid. In this catabolic pathway, adenine nucleotides can still be maintained in the muscle cell by three synthetic pathways: 1) from IMP via the purine nucleotide cycle (PNC); 2) from hypoxanthine via the purine salvage pathway; and 3) by *de novo* synthesis (for review see Tullson and Terjung, 1991). The former process involves the reamination of IMP to AMP, but does not occur to any significant degree during intense work conditions (Meyer and Terjung, 1980). The latter 2 processes are comparatively slow and expend comparatively larger amounts of energy, and thus are not favourable during exercise. Although some AN can be recovered, the appearance of hypoxanthine and uric acid in plasma is indicative of nucleotide loss from the muscle as a whole (Sewell and Harris, 1992). Thus plasma NH_3, hypoxanthine and uric acid are commonly used as markers of intramuscular AN loss. Tullson et al. (1995) measured the appearance of such purines in the plasma during a 4 min high-intensity cycle, and calculated that they accounted for 82% of the decrease in total AN that was measured.

During exercise (especially high-intensity exercise) it is optimal to maintain ATP levels to fuel ongoing contraction, and prevent AN degradation. It is possible that the hydrolysis products of AN degradation could have an indirect influence on muscle function (Sahlin, 1992). Indeed, a review by Banister and Cameron (1990) described that high

concentrations of NH_3 are thought to be neurotoxic, and exercise-induced hyperammonemia may be involved in the mechanism of muscle fatigue by its effect on the central nervous system (e.g. motor incoordination). Therefore, comparatively less NH_3 release into the blood at a given exercise intensity could be beneficial to performance.

2.3.2 The influence of exercise on adenine nucleotide degradation

Maximal exercise has been shown to cause an acute decrease in total muscle nucleotides, due to the inability of the skeletal muscle to match ATP degradation with ATP resynthesis. As discussed previously, studies have reported decreases of between 15% and 32% of resting ATP or AN levels immediately after exercise (Cheetham et al., 1986; Gaitanos et al., 1993; Sahlin and Broberg, 1990; Tullson et al., 1995). Variation between studies in the reported "loss" of AN appears to be related to different intensities and duration of exercise, and also will be dependent on the many metabolic factors that regulate AN degradation.

The extent of AN degradation and subsequent loss from muscle cells is dependent on the regulation of the pathway, and this occurs predominantly at the level of AMP deamination. At rest, AMP deaminase is most probably inhibited by the presence of P_i (Sahlin and Broberg, 1990). It is activated by increased free ADP and AMP concentrations, hydrogen ion (H^+) accumulation, and thus a lowering of intramuscular pH (Sahlin and Broberg, 1990). Although the acidotic activation of AMP deaminase is an important mechanism in the enhanced rate of AN degradation during exercise (Sewell and Harris, 1992), the degree of acidosis is not a prerequisite for increased AMP deaminase activity and subsequent AN degradation (Dudley and Terjung, 1985b; Sahlin and Broberg, 1990). Sahlin and Broberg (1990) propose that AMP deaminase activity will be stimulated in any situation where ADP rephosphorylation to ATP is inhibited or limited. This includes conditions such as high-intensity exercise, glycogen depletion, and low aerobic capacity (such as the untrained state). In addition, the control of AMP deamination varies between the fibre types (see section 2.3.3). There is also evidence to suggest that the initial (pre-

exercise) muscle ATP concentration will influence the extent of its degradation. Green et al. (1987) examining the decrease in ATP concentration following multiple 60 s cycle sprint bouts showed that the greater the ATP concentration before the sprint bout, the greater the amount of ATP loss during that bout.

2.3.3 The influence of fibre type composition on adenine nucleotide degradation

The extent of AN degradation is influenced by muscle fibre type composition. Although IMP accumulates from AMP deamination in all fibre types, concentrations at fatigue are twice as high in fast-twitch fibres (Sahlin and Broberg, 1990). Using an animal model, Meyer and Terjung (1980) showed that AMP deamination was initiated at lower workrates in fast-twitch white fibres (glycolytic) as compared to fast-twitch red fibres (oxidative-glycolytic). Also, in a study on contracting skeletal muscle in rats, Dudley and Terjung (1985a) found that the activation of AMP deaminase in fast-twitch red fibres could be attributed to the increase in free AMP substrate alone; but the activation in fast-twitch white fibres was due to factors other than the increase in substrate. Both of these separate findings imply that the control properties of this enzyme varies between fibre types.

The activity of AMP deaminase is almost three-fold higher in fast-twitch white fibres (Tullson et al., 1990). Dudley and Terjung (1985a) showed a decrease in AN of up to 50% in fast-twitch white fibres, while fast-twitch red fibres showed little depletion during contractions. The greater AN catabolism evident in fast-twitch white fibres appears to result from the inherent metabolic capability of the individual muscle fibres (including its activity and control), and the recruitment of fibres (Tullson et al., 1990; Tullson and Terjung, 1991).

Although the majority of studies examining fibre type and AN degradation have made use of the animal model, there are a few human studies as well. Greenhaff et al. (1994b) reported a decrease in ATP concentration of 3.5 mmol/kg dm in Type I, and 5.0 mmol/kg

dm in Type II fibres during a 30 s maximal treadmill sprint. This difference was however, not significant.

Thus it is apparent that during a bout of high-intensity exercise where fast-twitch fibres are maximally recruited (Burke and Edgerton, 1975; Vollestad et al., 1984), AN loss will be larger when compared to submaximal exercise. Also, it is possible that those athletes with a greater percentage of fast-twitch fibres will be more predisposed to AN degradation and loss. During high-intensity intermittent training - which is characterised by repeated periods of AN degradation and loss on consecutive days of training - this predisposition to lose AN is likely to be exacerbated. In addition, it has been shown that Type II (fast-twitch) fibres have a slower *de novo* synthesis rate than Type I (slow-twitch) fibres (Tullson et al., 1988). The question of whether *de novo* synthesis of AN restores TAN levels appropriately will discussed in Section 2.5.2.

2.3.4 The influence of exercise training on adenine nucleotide degradation

Hypothetically, the correlation between *de novo* synthesis rate and muscle oxidative capacity may imply that endurance training (which increases mitochondrial content) may also increase the capacity for *de novo* synthesis and consequently attenuate the loss in AN from exercise. As indirect support for this theory, Tullson and Terjung (1991) report that endurance training decreases the extent of AMP deamination.

Similarly, AMP deaminase activity is significantly reduced following high-intensity training (Hellsten-Westing et al., 1993a). There is also evidence to suggest that both IMP and NH_3 accumulation are reduced following high-intensity training (Tullson and Terjung, 1991). These findings suggest that the activity of AMP deaminase has decreased following training. Indeed, in a study by Hellsten-Westing et al. (1993a) after a 2 min maximal cycle test, AMP deaminase activity was markedly reduced following 6 wks of sprint training. This study also described lower plasma accumulation of hypoxanthine and uric acid during the highest intensity of a 2 min maximal test after training.

It is also highly likely that high-intensity training results in a more sustained decrease in AN stores which is not immediately recoverable (Green et al., 1987; Hellsten-Westing et al., 1993b; Stathis et al., 1994). This will be discussed in more detail in Section 2.5.2.

2.3.5 The influence of other metabolic pathways on adenine nucleotide degradation

During exercise, the rise in ADP and AMP concentrations are potent stimulators of the activation of both AMP deaminase and glycolytic enzymes, especially PFK. The relationship between the accumulation of lactate and NH_3 in the muscle and plasma suggests that the increase in AMP deamination is directly activated by H^+ associated with lactate formation (Sahlin and Broberg, 1990). In a study on trained thoroughbred horses, Sewell and Harris (1992) found that above a blood lactate concentration of 15 mmol/l, the relationship between plasma NH_3 accumulation and blood lactate concentration was linear, and this relationship was similar to that between muscle IMP concentration and muscle lactate. The authors suggest that this described a threshold to AN degradation which was probably related to a sudden increase in AMP deaminase activity.

It has also been shown that IMP formation is inversely related to muscle glycogen level (Sahlin and Broberg, 1990). A low initial muscle glycogen content prior to exercise to exhaustion at 70% $VO_{2\,max}$ resulted in a 5-fold greater increase in NH_3 released from the muscle to the plasma (Broberg and Sahlin, 1989). Based on these findings, the authors suggest that during prolonged exercise to exhaustion, a shortage of carbohydrate substrate results in an increase in AMP deaminase activity. According to Lowenstein et al. (1972) the contribution of glutamate deamination to NH_3 production is very low during high-intensity exercise. However, this may not be the case during such exercise in a state of partial glycogen depletion, and thus ought to be considered.

2.3.6 Conclusion

Exercise, especially high-intensity exercise, results in the degradation and loss of adenine nucleotides. The extent of this loss is dependent on a variety of factors including: the exercise type, intensity and duration; fibre type composition; state of training; and the substrate available for energy production (e.g. glycogen). The functions of creatine and phosphocreatine are intimately related to adenine nucleotide metabolism, and the importance of this link will become more clear as the effect of creatine supplementation on metabolism during exercise is discussed.

2.4 Creatine supplementation and exercise

The idea of creatine supplementation is not entirely new. Studies in the 1960's and 1970's examined the effect of altering creatine feeding to better understand creatine metabolism, pool size and turnover in man, and reference is even made to a study performed as early as 1926 (Chanutin, 1926). More recently, Crim et al. (1976) fed various doses of creatine precursors (arginine and glycine) to healthy men, and observed that the administration of dietary amino acid precursors can increase creatine synthetic rate. Gyrate atrophy of the choroid and retina is a disease characterised by impaired creatine biosynthesis. Sipila et al. (1981) reported the successful treatment of such patients by administration of 1.5 g creatine/day for a year. In summary, these data showed the following which is of relevance to this study: 1) the turnover rate of creatine is approximately 2 g per day in an average 70 kg male; 2) up to 95% of the body creatine pool is distributed in the skeletal muscle; and most interestingly, 3) the size of the body creatine pool can be influenced by the dietary creatine intake.

The notion of creatine supplementation as an ergogenic aid to exercise performance, however, has only received scientific interest very recently. Also, the area of exercise metabolism concerning the effect of nutritional supplementation on short-duration high-

intensity exercise capacity has tended to be overshadowed by the study of nutritional aids to endurance performance. This is surprising given that many sports events rely heavily on the "high-energy phosphate" energy pathways to resynthesise ATP and thus provide energy for muscular activity. This is the case in most team sports where exercise intensity varies and is characterised by bursts of maximal activity. In addition, athletes participating in endurance events also increase their exercise intensity intermittently, particularly during the final 30 seconds of a race. Although phosphocreatine is not a primary fuel source for the entire race, it may be of significant secondary importance during these periods of increased intensity.

Since 1992, there has been a noticeable increase in the study of the combination of creatine supplementation and high-intensity exercise.

2.4.1 The effect of creatine supplementation on muscle total creatine stores

A landmark study by Harris et al. (1992) showed that an oral supplementation regimen of 5 g creatine monohydrate four to six times a day for more than two days resulted in a significant increase in total muscle creatine content. The 5 g dose was chosen as it elicited a plasma creatine concentration well in excess of 500 μmol/l within an hour of ingestion, and repeated 5 g doses maintained this high level. Subsequently, most supplementation studies have followed a similar dosage protocol. Greenhaff (1995) suggests that low dose supplementation over a longer period (e.g. 3 g/day for 1 month) is not as effective in raising muscle creatine levels as the 5 g/day protocol. In addition, it appears that following the 5 g/day regimen, a maintenance intake of 2 g/day will maintain stores at the high (loaded) level. The "wash-out period" or natural decline of creatine from the muscle following loading is currently being investigated. Odland et al. (1994A) used only 14 days as a washout period between 3 days of placebo or creatine ingestion in a cross-over trial apparently without basing this choice on scientific evidence. Lemon et al. (1995A) used a 5 week washout period and reported that one subject's TCr content was still elevated at

this time, suggesting that 5 weeks may not be sufficient time for TCr levels to return to baseline in all cases.

Harris et al. (1992) showed a mean increase in muscle creatine content from 127 mmol/kg dm to 149 mmol/kg dm in 17 subjects (20% increase). Similarly, Greenhaff et al. (1994A) showed a 19% (~ 25 mmol/kg dm) increase in TCr stores following 5 days of creatine supplementation; and in another study Greenhaff et al. (1994a) reported that TCr content increased by approximately 15% after creatine supplementation. Also, preliminary results from 6 subjects who ingested 20 g of creatine per day for 6 days showed a mean increase in resting TCr of 25 mmol/kg dm (Soderland et al., 1994A). In Harris et al.'s (1992) study only 20% of the observed increase was in the form of phosphocreatine. In contrast, Odland et al. (1994A) found that only free creatine concentration was significantly elevated after 3 days of creatine supplementation while PCr and TCr levels were not changed. Creatine uptake into the muscle appears to be greatest within the first 2 days of beginning supplementation, and by the 3rd day, 68% of the ingested dose was excreted in the urine (Harris et al., 1992), suggesting that there is a limitation to the extent of creatine uptake.

In Harris et al.'s study (1992), there was noticeable variance between subjects in the extent to which they "loaded" with creatine. This is supported by Greenhaff et al. (1994a) who actually divided their analysis into a subgroup of "responders" (those subjects whose TCr content increased greater than 15%) and "nonresponders" (those subjects whose TCr content increased less than 10%) for further analysis. This observation was also noted by Lemon et al. (1995A) who, based on ^{31}P magnetic resonance spectroscopic (NMR) measurement of PCr, reported a prevalence of "nonresponders" to supplementation, although they do not clarify whether their classification of a "nonresponder" is based on 1) a lack of increase in PCr, 2) a lack of gain in mass, or 3) a lack of improvement in the performance test. Also, since ^{31}P NMR can only quantify PCr concentration, the lack of effect may be related to a lack of phosphorylation of the creatine taken up by the muscle.

Several factors are thought to affect this variance, although supporting data is still limited by relatively few studies and low subject numbers for some of the factors. Firstly, Harris et al. (1992) showed that in 5 subjects who performed one hour of hard one-legged *exercise* every day, the increase in TCr was significantly greater in the exercised leg than that observed in the sedentary leg. The extent of creatine uptake in athletes supplemented during *training* has not been investigated. Secondly, the greatest increases tend to occur in the subjects who had the lowest *initial muscle creatine contents*, especially those below 120 mmol/kg dm (Greenhaff et al., 1994a; Harris et al., 1992). In addition, there appears to be an upper limit to muscle creatine content in the region of 150 - 160 mmol/kg dm; which would imply that any loading effect of supplementation will depend in some cases on resting (pre-supplementation) creatine content (Greenhaff et al., 1994a; Harris et al., 1992). The concept of tight control of muscle creatine content is shown in a study by Fry and Morales (1980) on skeletal muscle culture. They manipulated the extracellular creatine culture medium over a 5700-fold range, and this only altered the intracellular creatine content by a factor of 20. Thirdly, the fact that dietary ingestion of both creatine and arginine and glycine precursors influences endogenous creatine synthesis, implies that an athlete's habitual diet may play a role in the extent to which he/she responds to creatine supplementation.

Another factor to consider is that the dose of creatine in all studies has been administered independently of body mass. An equal dose for all athletes may not be ideal to maximise loading, since elite athletes may range in body mass between 40 kg (for a gymnast for example) to over 200 kg (for a weight lifter). Also, although no studies to date have reported any harmful side-effects of supplementation, it may be of concern that the recommended loading dose is not set per kg body mass. In a study on 50 subjects between the ages of 19 and 85 yrs, Forsberg et al. (1991) reported that skeletal muscle TCr in relation to alkali-soluble protein was 1) significantly higher in females than males; and 2) not different between the ages .

2.4.2 The effect of creatine supplementation on body mass

Few studies have examined the effect of creatine supplementation on body mass closely. After creatine administration (20 g/day) for at least 5 days a mean increase in body mass of 1.3 kg, 1.8 kg, 1.1 kg, 1.0 kg, 1.5 kg and 1.6 kg was observed by Lemon et al. (1995A), Viru et al. (1993), Balsom et al. (1993), Stroud et al. (1994), Soderland et al. (1994A) and Greenhaff et al. (1994a) respectively. The exact mechanism by which this occurs is not clear. Balsom et al. (1993) suggest that part of the increase in body mass may be due to an increase in total body water content. This is likely since creatine is highly charged, therefore to keep the sarcoplasmic osmolality constant when creatine is absorbed, the cells will tend to gain additional water. A part of the gain in mass may also be due to a form of structural change at the myofibrillar level, possibly by increased contractile protein synthesis (Balsom et al., 1993). The theory of increase in protein synthesis is indirectly supported by Meyer et al. (1986) who report a decrease in fast twitch fibre diameter in creatine depleted rats, and similarly, Sipila et al. (1981) report an increase in fast twitch fibre diameter in patients fed creatine for the treatment of gyrate atrophy. In contrast, although earlier studies (Ingwall et al., 1972; Ingwall et al., 1974) indicated that creatine may increase the rate of myofibrillar protein synthesis in muscle tissue culture, more recently, the same group of researchers failed to confirm this phenomenon - despite the use of more up-to-date analysis techniques (Fry and Morales, 1980). At this time, the effect of creatine administration on body mass still needs further clarification.

2.4.3 The effect of creatine supplementation on exercise performance

An ergogenic effect of creatine supplementation on exercise may be to either improve peak performance or to improve resistance to fatigue. Several studies have investigated the effects of creatine loading on various types of exercise (Almada et al., 1995A; Balsom et al., 1993a; Birch et al., 1994; Burke et al., 1995A; Cooke et al., 1995; Earnest et al.,

1995A; Greenhaff et al., 1993; Grindstaff et al., 1995A; Hall et al., 1995A; Jacobs et al., 1995A; Lemon et al., 1995A; Odland et al., 1994A; Redondo et al., 1995A; Viru et al., 1993). However, few of these studies actually quantified whether or not there was a significant increase in muscle creatine content. It was assumed to occur, but whether this is actually always significant needs further evaluation. Thus, changes in performance, or a lack of change in performance should not be overinterpreted without evidence of the extent of creatine loading.

In a study by Greenhaff et al. (1993), subjects performed 5 bouts of 30 maximal isokinetic knee extensions (1 min recovery) before and after creatine or placebo ingestion. After creatine ingestion, peak torque production was significantly increased throughout bouts 2, 3, and 4; and at least part of bouts 1 and 5. Although muscle TCr was not directly measured by muscle biopsy sampling, or changes in body mass reported, the authors suggested that increasing muscle TCr increases both the amount of PCr available for contraction and also the creatine available for PCr resynthesis during exercise and/or recovery. They postulated that this increased rate of PCr resynthesis from mitochondrial ATP is due to increased efficiency of the PCr-Cr shuttle (thus suggesting that under normal conditions creatine availability may have a limiting role in the resynthesis of PCr). Another mechanism they mentioned that may contribute toward the enhanced performance with creatine ingestion, is increased buffering capacity.

Lemon et al. (1995A), using a similar, though longer exercise protocol (20 bouts of 30 s isometric ankle extensions with 16 s recovery) also found an ergogenic effect of creatine supplementation on the maximum force generated and the total integrated force (in the 10 min of exercise). During the creatine supplementation part of this cross-over design, subjects increased their mass (1.3 $\pm$ 0.3 kg) and increased their PCr/ATP ratio by 8%. One subject did not respond to creatine supplementation and thus was excluded from analysis. The authors do not clarify whether the subject was classified as a non-responder based on 1) a lack of increase in muscle PCr, 2) a lack of increase in body mass, or 3) a lack of improvement in performance.

Balsom et al. (1993a) examined the effect of 6 days of creatine supplementation on resistance to fatigue. After creatine ingestion, they found a significantly smaller decline in work output from baseline in 10 x 6 s bouts of high-intensity cycling at 140 revolutions per min (rpm) with 30 s passive rest after creatine ingestion, while the placebo group's performance was unchanged. Interestingly, no ergogenic effect was observed in resistance to fatigue (decline in work output) in the exercise test at 130 rpm, perhaps because the exercise intensity was lower (820 W vs. 882 W at 140 rpm). Despite this lack of significant effect on performance, the exercise test at 130 rpm did result in a significant alteration in metabolism which will be discussed in more detail in sections 2.4.5, and 2.4.6.

Another laboratory study (Birch et al., 1994) investigated the effect of 5 days of creatine or placebo ingestion on 3 bouts of 30 s maximal isokinetic cycling (with 4 min passive recovery). They found that peak power output, mean power output and total work output in the first 2 of the 3 bouts was significantly increased in their supplemented group. In contrast to the studies showing an ergogenic effect in the latter bouts of intermittent exercise (Greenhaff et al., 1993; Balsom et al., 1993a; Viru et al., 1993), in this study no effect of supplementation was observed in the 3rd exercise bout. The authors were also surprised at this finding, and suggested that the subjects' fatigue at that stage of the exercise was not related to energy substrate availability. This is possible as the decline in power during each cycle bout was approximately 11%, while McCartney et al. (1986) reported a decline in power in the order of 20% during similar exercise in trained subjects. This suggests that the subjects in Birch et al.'s study (1994) may not have been particularly well trained and thus more susceptible to muscle contractile fatigue due to metabolite accumulation as opposed to fatigue due to substrate limitation. Also the 4 min recovery period was noticeably larger than that used in the other creatine studies. The potential significance of this will be discussed in Section 2.4.4.

Following 6 days of creatine ingestion of 30 g/day, Viru et al. (1993) found a significant decrease in running time in the last bout of four 300 m (-0.7 s) and four 1000 m (-5.5 s)

runs (with 4, and 3 min rest intervals respectively). This finding is interesting in that the previous studies had only shown an ergogenic effect in exercise of very short duration (bouts of up to 30 s), while a 1000 m run lasting almost 2 mins is comparatively long and is considered a middle-distance event. During these exercise bouts, the heart-rate response was lower (statistical significance was not described) throughout the testing in the creatine loaded group despite the improved performance times. The authors suggested that there were enhanced oxidative processes in the skeletal muscle and myocardium, especially during the recovery periods, but this interpretation remains to be tested by metabolic methods.

It is apparent that an ergogenic effect is either evident in improved resistance to fatigue or improved peak performance, and not both. In Birch et al.'s study (1994), despite finding an increase in absolute power in each of the two first bouts following supplementation, the decline in power between bouts was very similar before and after supplementation and between the placebo and creatine group. Lemon et al.(1995A) also failed to show that creatine ingestion had an effect on the rate of force decline during the repeated exercise bouts, while maximum force values did improve. Thus, if there is a sufficient rest period, the rate of fatigue appears to be more a function of the exercise rather than the maximum power generated or the maximum TCr content. The improved resistance to fatigue and lack of improvement in peak power observed by Balsom et al. (1993a) was probably due to the limit imposed on peak power by having to cycle at prescribed revolutions per min. In another study (Earnest et al., 1995A), peak anaerobic power (peak power in a 5 s period) also did not improve in 3 bouts of 30 s Wingate tests (5 min recovery); but an ergogenic effect of supplementation was evident in anaerobic capacity (total work in 30 s) which was greater in all 3 bouts of exercise after 14 days of creatine supplementation.

Jacobs et al. (1995A) examined the effect of 5 days of creatine supplementation on maximal accumulated oxygen deficit (MAOD) - which is said to be a reflection of "anaerobic" exercise capacity. A significant ergogenic effect of creatine was evident in both the cycle time to exhaustion at 125% $VO_{2\,max}$ and the calculated MAOD, while the

placebo group's performance and MAOD was unchanged. A noteworthy observation they made was that the enhanced MAOD was still elevated 7 days after the cessation of creatine treatment.

Another study (Hall et al., 1995A) also investigated an indicator of anaerobic work capacity (W'). The linear relationship: $W = W' + CP(t)$ (where W, total work; W', the y-intercept; CP, critical power; and t, time to exhaustion) was tested by subjects performing 4 bouts of cycle ergometry at work-rates that would elicit fatigue between 1 and 10 mins, before and after creatine or placebo supplementation. Their results indicated that creatine ingestion increased W' ("anaerobic" capacity) but did not affect CP (which has been shown to relate to "aerobic" metabolic processes).

In contrast to the studies discussed above, a few studies have failed to show any ergogenic effect of creatine supplementation. It should be borne in mind that none of the following studies actually quantified the change in muscle TCr content. It is possible that the lack of ergogenic effect may be a result of insignificant creatine loading.

In a study by Cooke et al. (1995), subjects performed two 15 s cycle sprints separated by 20 min of rest, before and after 5 days of either creatine or placebo ingestion. No differences were observed in peak power, total work or fatigue index between or within the groups. There are 2 noticeable differences between this study and the others discussed previously. Firstly, the rest interval of 20 min is at least 5 times longer than any study showing an ergogenic effect of supplementation. This is particularly relevant when considering that the ergogenic effect of creatine loading may be related to enhanced ATP and PCr resynthesis, and a long recovery period would negate this effect (See Section 2.4.4). Secondly, the subjects in Cooke et al.'s study (1995) were untrained and were requested not to begin a structured exercise program during the trial. This may be significant since Harris et al. (1992) noted that exercise during supplementation augmented creatine uptake by the muscle. Although the change in muscle TCr was not directly measured, the authors acknowledged that there is question as to whether creatine

loading actually increases muscle PCr significantly. They suggested that an ergogenic effect on a single bout of high-intensity exercise may be dependent on the increase in PCr alone and not free creatine as the latter would not benefit the cell with an enhanced phosphoryl transfer potential. Cooke et al. (1995) concluded that creatine supplementation does not aid this type of exercise in untrained men.

Odland et al. (1994A) examined the effect of 3 days of creatine or placebo supplementation on a single bout of maximal cycling for 30 s (Wingate test). Despite an increase in skeletal muscle free creatine content, performance indices of power and fatigue were not changed. This lack of ergogenic effect may be related to the fact that only one bout of exercise was performed. As suggested by several studies showing an ergogenic effect of supplementation, the benefit only becomes apparent in the later stages of intermittent exercise.

In a field investigation, subjects performed 3 x 60 m sprints before and after placebo or creatine supplementation for 5 days (25 g/day) (Redondo et al., 1995A). There was no significant difference in running velocity between the groups before or after supplementation. In addition, in the event that an ergogenic effect only occurred in the *latter* stages of the run, the 60 m was divided into 3 parts for the analysis i.e. 20-30 m; 40-50 m; and 50- 60 m, but this still did not show any performance improvement with creatine supplementation. Similarly, Burke et al. (1995A) failed to show any enhancement in 25 m, 50 m, and 100 m sprint swim performance, or in a 10 s maximal cycle ergometry test, following 5 days of creatine or placebo supplementation in a group of 32 elite swimmers.

In a study by Grindstaff et al. (1995A) resistance trained males performed 5 x 15 maximal concentric repetitions on an isokinetic bench press and upright squat machine (60 s recovery) after 7 days of taking either maltodextrin or a mixed nutritional supplement containing 20 g of creatine per day. The only variable to improve significantly (6%) after supplementation with creatine was the absolute peak power generated in the bench press

test. No improvement was evident in peak power in the upright squat test. Also, average power, average force, peak force, total work, and all of these variables relative to body mass were unchanged in both tests. The authors conclude that creatine supplementation has limited effects on muscle endurance during intense, relatively slow-velocity resistance performance. In an extension of Grindstaff et al.'s study (1995A), Almada et al. (1995A) continued the same subjects' supplementation during 28 days of their normal resistance training. Although both groups showed an improvement in performance after the training, there was no difference between the placebo and creatine group for any of the variables measured. This is a novel finding as no other studies have examined the effect of supplementation during a period of high-intensity training. The results, however, should not be over-interpreted as, again, there was no muscle data to substantiate the extent of creatine loading that took place.

Although the majority of the studies on creatine supplementation have been performed using short-duration high-intensity exercise, a few studies have examined its effect on endurance performance. In a study by Balsom et al. (1993b) subjects performed a treadmill run at 120 % $VO_{2\,max}$ to exhaustion, and a 6 km terrain run before and after either creatine or placebo ingestion for 6 days. Contrary to expectation, after supplementation the creatine group showed a significant decrement in their terrain run performance, while the performance of the placebo group remained unchanged. The authors suggested that this finding may be related to the increase in body mass observed in the creatine group. Also, there was no change in the treadmill run in both groups. Stroud et al. (1994) examined the effect of 5 days of creatine supplementation on metabolic parameters during steady-state incremental treadmill run. There was no measurable effect of supplementation on respiratory gas exchange, blood lactate concentration, or heart-rate during and after the submaximal run.

2.4.4 The effect creatine supplementation on the resynthesis of PCr

During maximal exercise, the depletion of muscle PCr stores is considered a significant factor in the limitation of force production and the onset of fatigue (Hultman et al., 1967). Consequently, the maintenance of these stores by continued and efficient PCr resynthesis is essential to sustain maximal exercise performance. The effect of creatine supplementation appears to be dependent on the extent of PCr hydrolysis during exercise and other factors which affect its resynthesis (discussed below). Two studies which reported improved performances only in the latter bouts of maximal exercise following creatine supplementation (Balsom et al., 1993a; Greenhaff et al., 1993) attributed their results to an increase in resynthesis of PCr during the recovery phase.

Greenhaff et al. (1994a) examined the resynthesis of PCr following 20 electrically evoked 1.6 s isometric quadriceps contractions with 1.6 s rest between each contraction before and after 5 days of creatine supplementation (20 g/day). They found that although PCr resynthesis during the 1st min of recovery was similar before and after supplementation, during the 2nd min of recovery, the mean rate of PCr resynthesis was 42% higher following supplementation. It was apparent that those subjects who demonstrated the greatest increase in TCr following supplementation, also showed the greatest increase in PCr resynthesis rate after exercise. Greenhaff et al. (1994a) proposed that the improvements in work capacity with creatine supplementation reported in other studies may indeed be attributable to an accelerated rate of PCr resynthesis during recovery between exercise bouts. This is possibly the result of the increased availability of creatine driving the creatine-kinase reaction toward increased PCr production or an improvement in the creatine-PCr shuttle function.

From their preliminary findings, Soderland et al. (1994A) reported that PCr concentrations immediately after five 6 s cylce sprints were higher after versus before creatine supplementation.

Therefore, it is possible that the ergogenic effect of creatine supplementation may be dependent on the recovery/rest interval between exercise bouts. An ergogenic effect of supplementation has been shown by Lemon et al. (1995A); Balsom et al. (1993a) and Greenhaff et al. (1993) who used 16 s, 30 s, and 60 s recovery intervals respectively. Such durations would coincide with the initial and steeper part of the exponential PCr resynthesis curve. However, after approximately 4 min, the advantage may be lost as the slope of the curve plateaus and sufficient time is provided for almost complete PCr resynthesis - and accordingly there would be less dependence on the rate of resynthesis as a limiting factor to performance. Birch et al. (1994) made use of a 4 min recovery period between 3 bouts of 30 s maximal isokinetic cycle sprints and failed to show any performance improvement in the latter bouts of the exercise. A lack of ergogesnic effect was also reported by Cooke et al. (1995), who used a 20 min recovery period between 2 bouts of 15 s sprint cycling.

2.4.5 The effect of creatine supplementation on lactate accumulation.

The effects of creatine supplementation on lactate production and acid-base homeostasis were also investigated in some of these trials. Most of the studies report that plasma lactate accumulation, or post-exercise lactate concentration is not affected by creatine supplementation (Birch et al., 1994; Greenhaff et al., 1993; Greenhaff et al., 1994a; Greenhaff et al., 1994A; Odland et al., 1994A). From their lactate findings, both Birch et al. (1994) and Greenhaff et al. (1993) suggest that glycogenolysis and/or glycolysis is not directly affected by creatine ingestion despite increases in work output. Greenhaff et al. (1993) suggested that the energy for increases in work output observed was probably supplied by sources other that glycogenolysis (considering that glycogenolysis and glycolysis would be maximally activated during this type of exercise). However, if the latter is not the case, then one could argue that the fact that lactate accumulation was similar despite an increase in work output may imply that creatine supplementation has resulted in a "down-regulation" of the glycogenolytic/glycolytic system at any specific workload.

In an analysis of all subjects, Greenhaff et al. (1994a) found that muscle lactate concentration after exercise was not significantly changed after creatine ingestion. However, in a sub-group (5 out of 8) of "responders" (those subjects who demonstrated a *marked* increase in muscle TCr following supplementation), Greenhaff et al.(1994a) reported a significant increase in muscle lactate concentration immediately after exercise of the same intensity after creatine loading. From this, they suggested that an explanation for the improvement in exercise performance could be that the contribution of anaerobic glycolysis to ATP production was enhanced after creatine supplementation. The authors did acknowledge that in this study muscle force production was not measured with sufficient precision for it to be accurately related to lactate accumulation.

In contrast, preliminary findings have shown that muscle lactate accumulation immediately following five 6 s cycle sprints, and post-exercise muscle lactate concentration, were significantly lower after creatine supplementation despite more work being performed (Soderland et al., 1994A). Similarly, Balsom et al. (1993a) described lower post-exercise blood lactate accumulation after supplementation (11 vs. 9 mmol/l, and 7 vs. 5 mmol/l pre vs. post-supplementation for each exercise intensity). This data led them to propose that there was a change in the source of energy production during the exercise. Despite more work being performed, non-oxidative glycolysis was apparently reduced. Their suggestion was that the "down-regulation" was due to the reduced accumulation of ADP and AMP rather than increased accumulation of inorganic phosphates (which would in fact "up-regulate" glycolysis).

At this stage, data regarding the effect of creatine supplementation on lactate accumulation is inconsistent; especially considering that exercise duration is likely to be a critical factor. Thus conclusions or interpretations are not indisputable.

2.4.6 The effect of creatine supplementation on adenine nucleotide metabolism.

The metabolic effect of creatine supplementation includes a decrease in plasma ammonia concentration during and after high-intensity exercise (Birch et al., 1994; Greenhaff et al., 1993). Greenhaff et al. (1993) reported that the change in plasma ammonia concentration from baseline at the beginning of exercise was significantly reduced in the last 2 bouts (of 5 bouts) of exercise. Birch et al. (1994) showed that plasma ammonia accumulation at 2 mins post-exercise was significantly lower after creatine supplementation (160 μmol/l vs.129 μmol/l). This reduction in NH_3 concentration occurred despite the subjects often performing more work. In contrast, Earnest et al. (1995A) reported that plasma ammonia accumulation 2 mins after 3 x 30 s bouts of cycling was unchanged following creatine supplementation. However, significantly more work was performed during these exercise bouts, thus the lack of change in NH_3 concentration at a higher intensity of exercise may be indicative of a decreased accumulation at the same intensity with creatine ingestion.

It has been suggested that the reduced plasma ammonia concentration represents an increase in the efficiency of rephosphorylation of ADP to ATP due to greater PCr availability (Greenhaff et al., 1993). In addition, this may imply that there was a decrease in the amount of muscle ATP degraded to IMP, and thus also a decreased potential to lose total nucleotide via further degradation to hypoxanthine and urate.

Balsom et al. (1993a) measured plasma hypoxanthine concentrations after exercise, and observed that peak values decreased significantly from a mean of 21 to a mean of 17 μmol/l after an exercise test where on average more work was performed following supplementation (n = 8). They explained that their finding was an indication there had been a reduction in adenine nucleotide degradation.

This theory was examined more closely in a study by Greenhaff et al. (1994A). The decrease of ATP during each of 2 bouts of 30 s maximal voluntary isokinetic exercise was measured immediately on cessation of exercise before and after creatine supplementation.

They reported that during the second bout of exercise, mean ATP reduction was 50% less following creatine ingestion despite subjects performing more work. From their preliminary findings, the authors suggested that the attenuation in ATP degradation with creatine supplementation is probably related to an improved maintenance of the rate of ATP resynthesis from ADP.

2.4.7 Conclusion

It is apparent that creatine supplementation may enhance athletic performance during some types of short, intermittent maximal or near-maximal exercise. Also, there is evidence to suggest that creatine supplementation has an effect on energy metabolism during exercise and recovery from exercise. There is noticeable variance in the extent to which performance and metabolic changes occur, and this may be related to the type, intensity, and recovery duration of the exercise tested. In addition, the extent of increase in muscle creatine content as a result of supplementation, and certain attributes of the athletes themselves (such as fibre type, or prior training) warrant consideration.

2.5 High-intensity intermittent (sprint) exercise

Few studies have examined the physiological responses to high-intensity intermittent (sprint) exercise, which is surprising as many sports such as soccer, hockey, and basketball, incorporate periods of high-intensity sprint bursts, and brief periods of active recovery. In addition, many endurance athletes make use of intermittent sprint training sessions to "peak" for competition or enhance their "power" energy systems.

The majority of the energy required for such high-intensity short duration exercise is provided through oxygen-independent energy pathways. Exercise lasting less than 10 seconds predominantly relies on the degradation of PCr, while energy during exercise of

up to 1 min is provided through both PCr breakdown, and glycolytic/glycogenolytic degradation of glucose and glycogen to lactate (Gaitanos et al., 1993). This was also shown in a study by Cheetham et al. (1986) which examined muscle metabolism during a maximal 30 s treadmill sprint. They calculated that the relative contribution to ATP production during the 30 s from glycolysis, PCr degradation, and ATP depletion, was 63%, 32%, and 5% respectively.

2.5.1 High-intensity intermittent (sprint) training

Research has shown that high-intensity training improves high-intensity exercise performance (Houston and Thomson, 1977; Linossier et al., 1993; Nevill et al., 1989; Nielson et al., 1994). Nevill et al. (1989) reported a 12% improvement in peak power output during a 30 s treadmill sprint after 8 weeks of sprint training. Similarly, in a 30 s Wingate cycle sprint test after 7 weeks of training, increases in peak power of up to 17% (Stathis et al., 1994) and even 25% (Linossier et al., 1993) have been reported. After 6 weeks of training, anaerobic capacity as represented by the maximal accumulated oxygen deficit during a 30 s sprint run has also been shown to improve with sprint training in the order of 10% (Medbo and Burgers, 1990) and up to 17% and 20% as shown by performance time to exhaustion in a steep treadmill run (Houston and Thomson, 1977; Roberts et al., 1982 respectively). Significant improvements in Sargent's jump performance and endurance at 50% of maximal voluntary contraction have been reported after 8 weeks of sprint training (Thorstensson et al., 1975). Variance in the ergogenic effect of sprint training will obviously depend on the training status of the subjects, the exact nature and duration of the training, and the performance variables that are used to quantify any change.

In contrast to conventional training studies that last more than 4 weeks, a significant increase in 5 s power output in a sprint cycle test has been shown in a group of subjects that performed intensive sprint training for only one week (Hellsten-Westing et al., 1993b).

The mechanisms for such performance improvements have not been extensively examined. Adaptations to training have been observed in a variety of physiological systems including purine and glycolytic metabolism, enzymatic activities, and cardiovascular changes. For the purposes of this review, only those pertaining to purine metabolism and factors that affect it, will be discussed.

2.5.2 The effect of high-intensity training on resting high energy phosphate stores

Creatine and phosphocreatine levels in the skeletal muscle are remarkably constant and do not appear to change with high-intensity training (Green et al., 1987; Hellsten-Westing et al., 1993b; Houston and Thomson, 1977; Linossier et al., 1993; Nevill et al., 1989; Stathis et al., 1994; Thorstensson et al., 1975). However, one study (Boobis et al., 1983A) reported that resting PCr concentration was significantly lower after 8 weeks of training. It should be noted that the relatively lower PCr content may just reflect that the muscle samples were taken with the subjects in a comparatively less "rested" state, or as discussed by Bangsbo et al. (1993), a low PCr may be due to its artefactual hydrolysis during freezing of the muscle sample. A more accurate reflection of the effect of training on PCr and Cr would be represented by total creatine content.

Results from studies that examined the effect of high-intensity training on resting muscle adenine nucleotide levels are less consistent. A few studies have shown that post-training levels of muscle ATP are significantly decreased. In one of these studies (Stathis et al., 1994), subjects trained 3 times per week for 7 weeks during which they increased the number of 30 s cycle sprint bouts performed each session from 3 to 10 (with 4 min recovery between bouts, and 3 min recovery in the last 2 weeks). Training resulted in a 19% and 18% reduction in resting muscle ATP and total adenine nucleotide (TAN) levels respectively. The authors suggested that this was likely to be the result of an imbalance between ATP degradation and resynthesis, and they attributed it to either the acute effect of the prior exercise bout; or to the chronic effect of the high-intensity training. The

observation that the ATP concentration did not return to pre-training levels after 48 to 72 hours of rest implied that the latter cause was more likely. Stathis et al. (1994) proposed that this may describe a training induced down-regulation in resting ATP concentration. Although such a training adaptation would seem to be a paradox, ATP concentration should not be seen as an energy store. It is not the size of the AN pool that is essential to its function but rather the relative ratio of ATP/ADP, both of which influence energy flow, that is important. At the onset of exercise a comparatively lower ATP concentration could result in earlier acceleration of ATP resynthesis or an amplification of the signal.

Stathis et al. (1994) suggested that, due to the repeated depletion of ATP levels during intermittent exercise (as shown by McCartney et al., 1986), there is probably a sustained elevation in IMP concentration during a sprint training session. Thus, repeated sprint training sessions provide a sustained environment of enhanced catabolism of IMP, which results in the diffusion of hypoxanthine and inosine from the muscle, and the subsequent and repeated loss of purine bases.

In this study, despite a lower resting ATP content after training, subjects significantly improved their 30 s cycle sprint performance. This raises the question of whether sprint performances would have been further improved had subjects begun their post-training test with a higher resting ATP concentration.

In a study by Green et al. (1987), subjects trained for 3 days performing a total of between 14 and 24 bouts of 60 s sprints (with 240 s recovery) per day. By the 3rd day, resting muscle ATP levels had dropped by 20% (5.03 to 4.03 mmol/kg wet weight). The authors suggested that this may have been a result of either increased adenine nucleotide loss due to increased IMP formation, or perhaps a stress induced malfunction of the creatine-phosphocreatine shuttle and consequent failure to replete ATP stores. If, however, the latter theory is correct, the failure to replete ATP stores would have resulted in an unusually high ADP concentration in the muscle - yet Green et al. (1987) reported that ADP levels were not significantly altered after exercise and training.

Hellsten-Westing et al. (1993b) also examined the effect of sprint (high-intensity intermittent) training on TAN levels. Both experimental groups underwent one week of intensive training on a cycle ergometer during which they performed fifteen 10 s maximal cycle sprints with 50 s rest between bouts twice per day. In addition, one group had trained 3 times per week for 6 weeks prior to the intensive week (performing one set of the aforementioned exercise per day). The group that trained for 6 weeks showed a decrease in TAN from a mean of 25.1 to 22.0 mmol/kg dm. The intense training week had no additional effect. The group that only trained intensely for one week showed a decrease in TAN levels from a mean of 25.1 to 19.4 mmol/kg dm. This decrease was still apparent 3 days after training had ceased. Analysis of plasma creatine kinase activity led the authors to conclude that high-intensity intermittent exercise training caused a decrease in resting levels of total muscle adenine nucleotides without a concomitant indication of muscle damage.

This finding and the work of Stathis et al. (1994) both imply that an adaptation or response to high-intensity intermittent training may include a reduction in resting TAN concentration. In addition, those subjects that were non-specifically trained showed a significantly greater decrease in TAN than those subjects who underwent the initial 6 weeks of training. This suggests that the extent of the reduction in TAN with training may be moderated by both the nature and duration of training, and prior exposure.

As was found by Stathis et al. (1994), Hellsten-Westing et al. (1993b) also reported improvements in total work performed (6% to 8% increase) during a training session after the 6 weeks of training despite lowered skeletal muscle TAN content.

In contrast to the work of Stathis et al. (1994), Green et al. (1987) and Hellsten-Westing et al. (1993b), a other sprint training studies have not shown a *decrease* in resting ATP levels 48 to 72 hours after the last training session. In a study by Nevill et al. (1989), subjects trained 4 times per week for 8 weeks (this included 2 days of 2 x 30 s treadmill

sprints with 10 min recovery; 1 day of 6 to 10 x 6 s sprints with 54 s recovery; and 1 day of 2 to 5 x 2 min runs at 110% $VO_{2\,max}$ with 5 min recovery). They reported that resting ATP concentration was slightly, but not significantly, lower after training. Another study that examined intermittent treadmill sprint training 3 to 4 times per week for 8 weeks was performed by Thorstensson et al. (1975). Subjects completed multiple 5 s sprints (25 to 55 s recovery), and over the 8 week period increased the number of repetitions (from 20 to 40), the speed (from 19 km/hr to 24 km/hr), and the incline of the run. Despite small subject numbers (n = 4), the authors reported that there was *no significant change* in resting ATP concentration after training. Similarly, no changes in resting ATP stores were found in subjects who performed intensive sprint training either 5 times per week for eight weeks (Boobis et al., 1983A) or 4 times per week for 7 weeks (Linossier et al., 1993).

On the other hand, there are two studies that show an increase in ATP levels following intense training. Houston and Thomson (1977) reported a 15% increase in resting ATP concentration (from 25 to 29 mmol/kg dm) after 6 weeks of intense intermittent hill running 4 times per week. The training protocol was quite varied and included: 3 x 60 s sprints (2 min recovery), 5 x 6 s sprints (24 s recovery), and 2 x 90 s sprints (3 min recovery). In this study, however, only 5 subjects were examined, and they were noticeably older (34 to 37 years) than those in the other studies. Eriksson et al. (1972) reported an *increase* in resting ATP concentration from 4.3 to 4.8 mmol/kg wwt. after training. Although the training employed was intermittent in nature, it was not strictly high-intensity as it included 15 to 20 min intermittent runs, some basketball and soccer games.

In summary, it is apparent that the literature is inconsistent regarding the effect of high-intensity training on resting muscle ATP stores. Both Stathis et al. (1994) and Hellsten-Westing et al. (1993b) suggest that the discrepancies are best explained by differences in the training protocols used in each study. The difference does not appear to be related to the recovery interval between exercise bouts. Those studies that reported an increase in ATP stores following training used rest periods from 24 s to 3 mins; those in studies that

reported no change in ATP stores used rest periods from 25 s to 10 mins; and lastly, those studies that report a decrease in ATP stores used rest intervals between 50 s and 4 mins.

The difference appears to be related more to the specificity of the exercise used. Those studies that showed a *decrease* in ATP concentration following training used training protocols that 1) were extremely strenuous, 2) made use of one type of exercise at maximal intensity, and 3) imposed a training overload by increasing repetitions of the exercise. In contrast, the other studies' training protocols incorporated a variety of exercise types and intensities.

2.5.3 The effect of high-intensity training on products of adenine nucleotide degradation, enzymes activities, and the activity of oxygen-independent glycolysis.

High-intensity intermittent exercise is characterised by large increases in plasma ammonia, hypoxanthine, and uric acid concentrations after exercise. There is evidence to suggest that high-intensity intermittent training results in an adaptation in purine nucleotide metabolism and hence alters the accumulation of such compounds.

The large increase in plasma ammonia concentration after high-intensity exercise is significantly attenuated with sprint training (Snow et al., 1992; Stathis et al., 1994). Snow et al. (1992) proposed that sprint training alters muscle NH_3 efflux and/or the removal of NH_3 from the plasma rather than a decrease in muscle NH_3 production. This argument, however, is based on the premise that ATP degradation during a bout of sprint exercise is unaltered by training (Nevill et al., 1989), but there is evidence to suggest the contrary. Stathis et al. (1994) report a 52% decrease in the extent of ATP degradation during a sprint bout after training. Thus there exists the possibility that the mechanism by which plasma NH_3 accumulation is decreased after training is related to decreased ATP degradation and subsequent ammonia production.

Plasma accumulation of both hypoxanthine and uric acid following a 2 min maximal exercise test has been shown to be significantly lower after 6 weeks of sprint training (Hellsten-Westing et al., 1993a). The authors proposed three reasons for the decrease in hypoxanthine concentration following training. Firstly, it may be due to an increase in the amount of intracellular hypoxanthine that is rephosphorylated to IMP. This explanation is probable as Hellsten-Westing et al. (1993a) also report that the activity of the enzyme responsible for this conversion (hypoxanthine phosphoribosyl transferase, HPRT) was higher after training. This would be a beneficial adaptation to training as it would reduce nucleotide loss from the muscle. The second explanation for the decrease in hypoxanthine accumulation is that less hypoxanthine was actually produced during the exercise. This explanation also seems feasible as the authors found that the activity of AMP deaminase was markedly decreased following training. This down-regulation would result in lower IMP and ammonia accumulation, thus explaining the decrease in degradation of IMP to purines. The third explanation is that after training, there was less dependence on adenine nucleotide degradation for the resynthesis of ATP at the same workload. This would be a result of an enhanced ability to resynthesise ATP via oxygen-independent glycolysis - and this theory is supported by their finding that PFK activity was increased after sprint training (Hellsten-Westing et al., 1993a).

Linossier et al. (1993) also proposed that sprint training results in an increase in the contribution to energy production from oxygen-independent glycolysis during high-intensity exercise. They reported a 20% increase in both PFK and lactate dehydrogenase (LDH) activities and a corresponding increase in post-exercise lactate accumulation after 7 weeks of sprint training. Even greater increases in PFK and LDH activities were described by Roberts et al. (1982) after a 5 week training period which included 3 to 4 sessions per week of multiple 200 m sprints. Stathis et al. (1994) suggested that the most likely source of an improved ATP resynthesis rate with sprint training is an enhanced glycolytic capacity. Also, based on estimations from changes in muscle metabolites, Nevill et al. (1989) reported that in a 30 s sprint after training, total ATP resynthesis from

anaerobic sources increased by 14%, and 20% of this increase was from anaerobic glycolysis.

There are, however, studies which do not support the theory that sprint training increases oxygen-independent glycolytic activity. Both Thorstensson et al. (1975) and Housten and Thomson (1977) failed to show an increase in LDH activity which was used as a marker of oxygen-independent glycolysis. Thorstensson et al. (1975) did, however, report an increase in the activities of myokinase (MK), adenylate kinase (AK) and creatine-phosphokinase (CPK) following the 8 weeks of sprint training - implying that the capacity for ATP turnover was higher following training. In contrast, Hellsten-Westing et al. (1993a) report that AK activity did not change after training. Inconsistencies apparent in the literature are probably due to differences in training regimens used in each study, and the extent to which the training stressed the phosphagen system alone, or the phosphagen and oxygen-independent glycolytic systems together.

When examining studies on the adaptations to sprint training, it should be borne in mind that adaptive responses are subject to the principle of specificity and individuality. This is shown in a study by Saubert et al. (1973) who used an animal model to examine the effect of 11 weeks of high-intensity intermittent sprint training on certain key glycolytic enzyme activities. Saubert et al. (1973) found that the soleus was the only muscle that showed an enzymatic adaptation to the sprint training (shown by increased phosphorylase and pyruvate kinase activities). The authors concluded that prior to training, the glycolytic capacity of this muscle was so low, and thus an adaptation was necessary to meet the functional and metabolic demands of the daily sprint exercise. Those muscles with an adequate glycolytic capacity prior to training did not improve this capacity further with training.

2.5.4 Conclusion

If high ATP degradation rates during sprint training result in an inability to completely restore the purine base loss during rest days, intramuscular ATP and TAN levels at rest will be reduced. It is unknown how long it takes for *de novo* synthesis to replete "chronically" depressed TAN levels since no studies have made measurements more than 3 days post-training.

High-intensity intermittent training results in adaptations that can attenuate a loss in adenine nucleotides which if prolonged and progressive could potentially affect performance or the ability to train efficiently. This is achieved through alteration in both absolute levels of purine compounds (resting ATP; and urate; hypoxanthine; and ammonia during exercise), and purine turnover as shown by enzyme activities (AMP deaminase; myokinase; creatine phosphokinase, HPRT). It is apparent that the rate of decrease in adenine nucleotide content of skeletal muscle resulting from the initial demands of high-intensity training may be slowed down by continued training.

This is an important practical consideration for the athlete in training. Most endurance athletes will "peak" for an event by increasing their exercise intensity and developing the "power" energy systems. It is at this point that strategies to optimise and prepare for this increase in training intensity need to be employed. It is not known whether the improvement in exercise performance following high-intensity training may be even greater if the muscle ATP pool could be maintained.

2.6 Summary and hypotheses: the effect of prior creatine supplementation on adenine nucleotide metabolism during sprint training

One of the issues raised in previous studies on creatine supplementation was that some subjects did not respond to supplementation and thus did not enhance their total creatine content significantly. The prevalence of "non-responders" and, possible reasons for this need to be examined before results from these studies can be applied to the greater sporting population with confidence.

It has been established that creatine supplementation enhances performance in high-intensity intermittent exercise (Balsom et al., 1993; Birch et al., 1994; Earnest et al., 1994A; Greenhaff et al., 1993; Hall et al., 1995A; Jacobs et al., 1995A; Lemon et al., 1995A; Viru et al., 1993). This ergogenic effect is most probably a result of enhanced control of adenine nucleotide metabolism. In addition, high-intensity intermittent training causes a decrease in adenine nucleotide stores (Green et al., 1987; Hellsten-Westing et al., 1993b; Stathis et al., 1994). An interesting question that arises from these separate short-term studies, is whether creatine administration during the acute phase of high intensity training can prevent the observed loss in muscle adenine nucleotide stores. The possible mechanism explaining any or all of the above hypotheses is that higher muscle TCr stores result in a better maintenance of the adenine nucleotide pool and its metabolism. If this is the case, performance improvements from high-intensity sprint training may be enhanced with creatine supplementation. To date, no study has investigated whether an increased availability of phosphocreatine does in fact improve the adaptation to high-intensity training.

This study will examine some of the questions raised above, namely:

i) whether skeletal muscle fibre type composition influences the efficacy of creatine loading in human skeletal muscle;

ii) whether the decrease in skeletal muscle adenine nucleotide content from sprint training is attenuated by creatine loading and maintenance; and,

iii) whether the effect of sprint training on both sprint and high-intensity endurance performance is improved with creatine loading.

CHAPTER 3:

GENERAL METHODS

3.1 Exercise performance tests

3.1.1 Peak sustained power output test

The peak sustained power output test (PSPO) according to the protocol of Hawley and Noakes (1992) involved an incremental cycle to volitional fatigue on a Lode cycle ergometer (Lode, Gronigen, Netherlands). The initial workload was 3.33 watts/kg body mass, followed by two 50 watt (W) increments, after which the increment in workload was 25 W until exhaustion. Each workload was maintained for 2 min 30 s. PSPO was calculated by the equation: $PSPO = W_{final} + (\Delta WL \times t / 150)$, where W_{final} is the last completed workload, and t is the time (s) that the final uncompleted workload was sustained, and ΔWL is the workload increment. Subjects were required to remain seated throughout the test. The test was terminated on indication of exhaustion from the subject, or when the pedal cadence dropped below 60 revolutions per min (rpm).

3.1.2 Wingate anaerobic power test

The Wingate anaerobic power test (WAT) was performed on a mechanically braked Monark cycle ergometer (Monark, Stockholm, Sweden), interfaced with an Apple microcomputer (Apple, Chicago, Illinois, USA). The ergometer was modified with racing handle-bars and cleat pedals. Subjects performed a standardised warm-up of 10 min of light cycling interspersed with some sprint bursts. The workload chosen for the well-trained subjects was 0.1 kilopond/kg body mass. The power output measurements were not corrected for inertia of the fly-wheel, but power measurements were not initiated until the cyclist attained unresisted acceleration of the fly-wheel. From this test, measurements of peak power, mean power, peak cadence, and fatigue rate were made. Peak cadence was measured by a photo-optic sensor on the side of the ergometer's wheel, and the other variables were calculated by the on-line computer program according to the following equations: peak power (W) = workload (kiloponds) x peak cadence; mean power (W) = (Σ power at each second) / 30; fatigue rate (W/s) = (peak power - power at t = 30 s) / 30.

3.1.3 1-hr Distance trial

During the 1-hr distance trial subjects were required to cycle as much distance as possible in an hour using self-selected workloads. The test was performed on a Kingcycle ergometer (Version 4.1, 1991, EDS Portaprompt Ltd, High Wycombe, Bucks). The advantage of this system is that it allows cyclists 1) to be tested in the laboratory, but under conditions that approximate road cycling more closely; and 2) to use their own bicycle. The reliability of this system was shown in a study by Keen et al. (1991A) who showed that the test retest correlation of maximal aerobic power tests performed on the Kingcycle was 0.98.

The bicycle was supported by resting the bottom bracket on an adjustable prop and attaching the front forks to the frame of the ergometer. The prop was adjusted to standardise the amount of rolling resistance applied to the rear wheel of the bicycle. Power was produced by the rolling of the rear tyre against a calibrated air brake and flywheel. The mass of the flywheel and the resistance of the air brake were engineered so as to simulate the inertial load experienced by a 70 kg cyclist and his/her bicycle. Power output and cycle revolutions per min were measured by a system of photo-optic cells. The ergometer system was linked to an IBM compatible PC which displayed and recorded data continuously.

Prior to each 1-hr test, the Kingcycle was calibrated to ensure that the results of tests were both valid and repeatable. Using a run down technique, the Kingcycle computer software calculated the extent to which the rolling resistance must be altered for the mass of the cyclist and his/her bicycle on the ergometer, and the adjustable prop was altered manually accordingly.

3.2 Blood sampling and analysis

For blood sampling, a Jelco teflon cannula was inserted into a forearm vein and attached to a 3-way stopcock. To maintain its patency, the cannula-stopcock unit was routinely flushed with heparinised saline (1 ml heparin in 100 ml saline) (Sabax Sodium Chloride 0.9% Sabax Ltd, Johannesburg, South Africa)(1 ml Heparin = 5000 units, Pularin, Allen, Wadeville, South Africa) after each blood sample was taken.

At each sampling time, a 2 ml blood sample was drawn and discarded in order to remove any saline that may have been present in the cannula-stopcock unit. Then a 4 ml blood sample was withdrawn and injected into a stoppered test tube containing EDTA. This was mixed and centrifuged (Sigma, Laborzentrifugen, GmbH, Germany) immediately for 6 min at 3000 rpm (~2200 g) at approximately 4° C. Directly after the trial some plasma from each sample was enzymatically assayed for plasma ammonia concentration using the prepackaged kit method (Boehringer Mannheim GmbH, Mannheim, Germany) and the remaining supernatant was stored at -20° C for later analysis of plasma lactate, urate and hypoxanthine concentrations. Lactate was analysed using a prepackaged kit method (Lactate Kit PAP, BioMerieux, Lyon, France), while hypoxanthine and urate were analysed by high performance liquid chromatography (HPLC).

3.2.1 HPLC analysis method for plasma urate and hypoxanthine

a.) Sample Extraction

A 100 µl volume of plasma sample was deproteinised in 100 µl of cold 0.6 M HPLC-grade perchloric acid (PCA) (BDH, Hipersolv, England) and then mixed on a rotary mixer. The solution was then centrifuged in an micro-centrifuge (Eppendorf, Geratebau, Germany) at 11500 rpm (~8500 g) for 4 min. A 150 µl aliquot of the supernatant was then neutralised with 9 µl of 2.5 M potassium carbonate (K_2CO_3, UnivAR, Saarchem Pty Ltd, Krugersdorp, SA). After this was thoroughly mixed, it was placed on crushed ice for

approximately 10 min to precipitate the potassium perchlorate salt. Following this, the solution was centrifuged as above for a further 4 min. Then, a 100 µl aliquot was mixed with 400 µl of the mobile phase phosphate buffer (see below). A small volume (20 µl) of this extracted sample solution was used for injection onto the HPLC column.

b.) Mobile Phase Buffer

The mobile phase consisted of a 50 mM potassium dihydrogen orthophosphate (KH_2PO_4) (BDH, Hipersolv, England) buffer which was made up with deionised water (> 14 MΩ). The pH of the buffer was 4.65. The buffer was filtered through a 0.45 µm nylon membrane filter (Supelco Inc., Bellafonte, PA) and degassed before use.

c.) Standard Solutions

Stock solutions of both hypoxanthine (1.521 mmol/l) and urate (3 mmol/l) were made up in advance of the analysis and were stored at - 20° C as frozen 1 ml aliquots. On each analysis day, new stock aliquots of each compound were thawed and used to make up fresh standard solutions. Two standard solutions were used and were made up to a volume of 1 ml with the mobile phase buffer: standard 1 (hypoxanthine = 50.19 µmol/l, urate = 456 µmol/l) and standard 2 (hypoxanthine = 19.77 µmol/l, urate = 306 µmol/l). The compound concentrations in the two standards were chosen to cover the range of hypoxanthine and urate concentrations typical of human plasma at rest and after high-intensity exercise.

In order to calculate the concentration of the sample, it was necessary to quantify the area under the peak of each standard. On each day, the final standard area of each compound was taken as the average of the two standard areas for that compound. The reproducibility of the standard areas between each run and each day was good and this is displayed in Table 1.

TABLE 1. Coefficient of variation (CV) in the peak area of hypoxanthine and urate within the standard runs on each day, and between the standard runs over all analysis days.

	Hypoxanthine	Urate
Mean within day CV	2.26 %	2.51 %
Between day CV	6.57 %	4.94 %

Since the coefficient of variation was better within each day than between days, each plasma sample concentration was calculated using the mean standard area of the day on which it was analysed.

d.) Analysis

After completion of all the exercie tests, samples were analysed in a random order on 11 consecutive days. The samples were analysed using a Gilson HPLC system (Gilson Medical Electronics, France) with Gilson software. The column was an Alphasil 5ODS (25 cm x 4.6 mm, HPLC Technology, Cheshire, UK). The Gilson 119 ultraviolet (UV) detector (Gilson Inc., Middleton, USA) was set at 249 nm. The chromatography was performed at ambient temperature and the flow-rate was 1 ml/min for the duration of the 10 min run-time. From the extracted sample solution, 20 µl was injected through a 20 µl loop in the injection port (Rheodyne 7161, California, USA). Data collection only commenced after 4 min because during the inital 4 min of the run a variety of intefering peaks eluted which were of no consequence to the run. From the beginning of data collection, the elution time of hypoxanthine was 4.27 ± 0.42 min, and that for urate was 2.45 ± 0.38 min. The analysis conditions included a peak width of 0.5 min, and peak sensitivity of 5%, and compounds were quantified according to peak area. Chromatograms of a standard and a muscle sample are presented in Appendix IV.

3.3 Muscle sampling and analysis

Muscle was sampled from vastus lateralis on 3 occasions using the percutaneous needle biopsy technique described by Bergstrom (1962), as modified by Evans et al. (1982). All biopsies were taken with the subject at rest, and at the same time of day.

Muscle biopsies were performed by a qualified and experienced medical doctor under sterile conditions. The biopsy site was cleaned, and 3 ml of 1% lignocaine (Intramed Pty Ltd, Port Elizabeth) was injected subcutaneously. Once the local anaesthetic had taken effect, a scalpel incision (±1 cm) to the level of the muscle was made. Following this, the biopsy needle was inserted into the muscle, and a sample was taken. The site was cleaned and dressed appropriately.

On removing the muscle sample from the biopsy needle, it was frozen in liquid nitrogen immediately. The estimated time lapse from removal to freezing was less than 5 s for the 2nd and 3rd biopsies, and less than 10 s for the 1st biopsy as a section of this sample was cut off to be used for fibre type analysis.

The muscle samples were initially stored at -80° C, and then freeze-dried for subsequent analysis of Cr, PCr, ATP, ADP, and AMP using HPLC. This analysis was based on the isocratic method for the simultaneous determination of creatine compounds and adenine nucleotides descibed by Sellevold et al. (1986) with modifications that will be described later (see section 3.3.2).

3.3.1 Fibre type analysis

Fibre type analysis was performed by the Pathology Department of Red Cross Hospital using the method described by Dubowitz (1985). The piece of muscle cut off was kept on ice for approximately 15 min, and then under a microscope was mounted on a slice of cork and prepared for fixing with a small amount of Tissue-Tek. The samples were frozen by

immersion and agitation in N-pentane pre-cooled to just above freezing point by liquid nitrogen. Samples were stored in air tight containers at - 80° C. Transverse sections (5 - 7 microns) were stained for ATPase activity at pH 9.4 for analysis of the 2-fibre classification system, and after preincubation at pH 4.3 and pH 4.6 for distinction of Type IIA and Type IIB fibres according to Brooke and Kaiser (1970). The fibre type proportions of approximately 150 Type I, Type IIA, and Type IIB muscle fibres differentiated by ATPase staining were measured using an IBAS Kontron Image analysis system.

3.3.2 HPLC analysis method for skeletal muscle creatine compounds and adenine nucleotides

a.) Sample Extraction

The freeze-dried muscle sample was dissected free of visible blood and connective tissue and then powdered. Approximately 5 mg of sample was extracted via graded homogenisation. This involved adding an initial 100 µl of cold 0.6 M HPLC-grade PCA (BDH, Hipersolv, England) to the sample powder in a glass vial, and homogenising for approximately 2 min or until a milky paste was obtained. An additional 900 µl of PCA was then added to wash off any traces of the homogenate solution on the glass plunger. The homogenate was poured into an eppendorf tube, mixed on a rotary mixer and then centrifuged (Eppendorf, Geratebau, Germany) at 11500 rpm (~8500 g) for 4 min. A 200 µl aliqout of the supernatant was then neutralised with 21 µl of 2.5 M K_2CO_3 (UnivAR, Saarchem Pty Ltd., Krugersdorp, SA). This was well mixed and placed on crushed ice for approximately 10 min to precipitate the potassium perchlorate salt. Following this, the solution was centrifuged as above for a further 4 min. From this, a 100 µl aliquot was mixed with 900 µl of the mobile phase phosphate buffer. A small volume (20 µl) of this extracted sample solution was used for injection onto the HPLC column.

b.) Mobile Phase Buffer

This analysis method is based on the isocratic method described by Sellevold et al. (1986). They prescribed using a phosphate (KH_2PO_4) concentration of 215 mM. From their study, it was shown that such a high salt concentration resulted in significantly decreased retention times of all compounds. In this study however, a buffer containing 215 mM phosphate did not give a distinct separation of compounds or a satisfactory baseline. Hence, through trial and error, a phosphate concentration of 90 mM was chosen. The increased retention time associated with lower salt concentrations in the mobile phase was overcome by altering the flow-rate in the later stages of the run.

The final mobile phase contained 90 mM KH_2PO_4 (BDH, Hipersolv, England); 2.3 mM tetrabutylammonium hydrogen sulphate (TBAHS) (TCI-Ace, Tokyo Kasei Kogyo Co Ltd, Tokyo); and 3.5% HPLC-grade acetonitrile (BDH, Hipersolv, England). After the addition of the acetonitrile, the pH was adjusted to 6.25 with 40% potassium hydroxide (KOH, BDH, Analar, Poole, England), and then the solution was made up to volume with deionised water (> 14 MΩ). The mobile phase was filtered through a 0.45 μm nylon membrane filter (Supelco Inc., Bellafonte, PA) and degassed before use.

c.) Standard Solutions

Stock solutions of all the compounds (ATP, ADP, AMP, PCr, Cr, Creatinine (Cn), urate, hypoxanthine (Hx) and nicotinamide adenosine dinucleotide (NAD)) were made up in advance of the analysis and were stored at - 20° C as individual 1 ml aliquots. On each analysis day, new stock aliquots of each compound were thawed and used to make up fresh standard solutions. Two standard solutions were used and were made up to a volume of 1 ml with the mobile phase buffer. The compound concentrations in the standards were chosen to cover the range of compound concentrations typical of human muscle at rest and after high-intensity exercise.

In order to calculate the concentration of the sample, it was necessary to quantify the area under the peak of the standard. Each day, this was taken as the average of the two

standard areas. The reproducibility of the standard areas between each run and each day was good and this is displayed in Table 2.

TABLE 2. Coefficient of variation (CV) in the peak height of PCr and Cr, and the peak area of ATP and ADP within the standard runs on each day, and between the standard runs over all analysis days.

	PCr	Cr	ATP	ADP
Mean within day CV	2.35 %	1.81 %	3.26 %	3.23 %
Between day CV	2.42 %	2.27 %	3.22 %	4.94 %

d.) Analysis

After completion of all the exercise tests, the muscle samples were analysed in a random order on 4 consecutive days. The samples were analysed using a Gilson HPLC system (Gilson Medical Electronics, France) with Gilson software. The column was an Alphasil 5ODS (25 cm x 4.6 mm) (HPLC Technology, Cheshire, UK). The Gilson 119 UV detector (Gilson Inc., Middleton, USA) was set to dual wavelength detection mode; that is 210 nm for the creatine compounds and 260 nm for the adenine nucelotides. The chromatography was performed at ambient temperature. The duration of the run was 20 mins. For the first 8 min the flow-rate was 0.6 ml/min, then over 1 min it was increased to 1.2 ml/min. This was maintained until the 19th min, afterwhich the flow-rate was reduced to baseline (0.6 ml/min) by the 20th min.

From the extracted sample solution, 20 µl was injected into the injection port (Rheodyne, 7161, California, USA). The elution times (min) of the compounds were as follows:

Cr = 4.64 ± 0.4; Cn = 5.23 ± 0.24; PCr = 5.71 ± 0.24;
Hx = 6.19 ± 0.24; Urate = 6.9 ± 0.34; NAD = 8.55 ± 0.37;
AMP = 9.61 ± 0.37; ADP = 11.89 ± 0.63; ATP = 14.9 ± 0.6.

Chromatograms of a standard and a muscle sample are presented in Appendix V.
In the analysis conditions, the peak width was set at 0.5 min and peak sensitivity at 5%. In the muscle sample runs an unidentified peak eluted immediately after that of creatine and intefered with its baseline. Consequently, the creatine compounds (PCr and Cr) were quantified according to peak height instead of peak area. Since PCr could be analysed using peak height or peak area, the PCr concentration in selected standard and sample runs was calculated from both peak area and peak height. These were found to be similar.

CHAPTER 4:

THE EFFECTS OF PRIOR ORAL CREATINE SUPPLEMENTATION ON PERFORMANCE AND METABOLISM AFTER 7 DAYS OF INTERMITTENT SPRINT CYCLE TRAINING.

4.1 Study Design

4.1.1 Subjects

Thirteen male endurance trained cyclists (with the ability to complete a 110 km cycle in under 3 hours) volunteered as subjects. Endurance trained subjects were chosen so that they had a baseline fitness level that was sufficient to withstand the overload of laboratory sprint training. The subjects were randomly, and in a double-blind fashion, assigned to an experimental group (those receiving a creatine supplement), or control group (those receiving a placebo). The nature and risks of the experimental procedures were explained (Appendix I), and written informed consent was obtained from the subjects (Appendix II). The study was approved by the Ethics and Research Committee of the University of Cape Town.

4.1.2 Experimental design

The laboratory trial spanned 22 days. All subjects were familiarised with the performance tests and the training protocol during the week prior to the experimental trial. Initially, subjects ingested a loading dose of the creatine supplement or placebo for 7 days, after which they ingested a maintenance dose for the remainder of the trial (see section 4.1.4). After supplement loading, subjects were required to do 3 exercise performance tests and a metabolic test. The metabolic test consisted of blood sampling during multiple sprint bouts in the laboratory (defined in more detail in section 4.1.6). The tests took place on 3 different days, each separated by a day of rest. After the week of sprint training, the metabolic and performance tests were repeated in a similar manner (Figure 1).

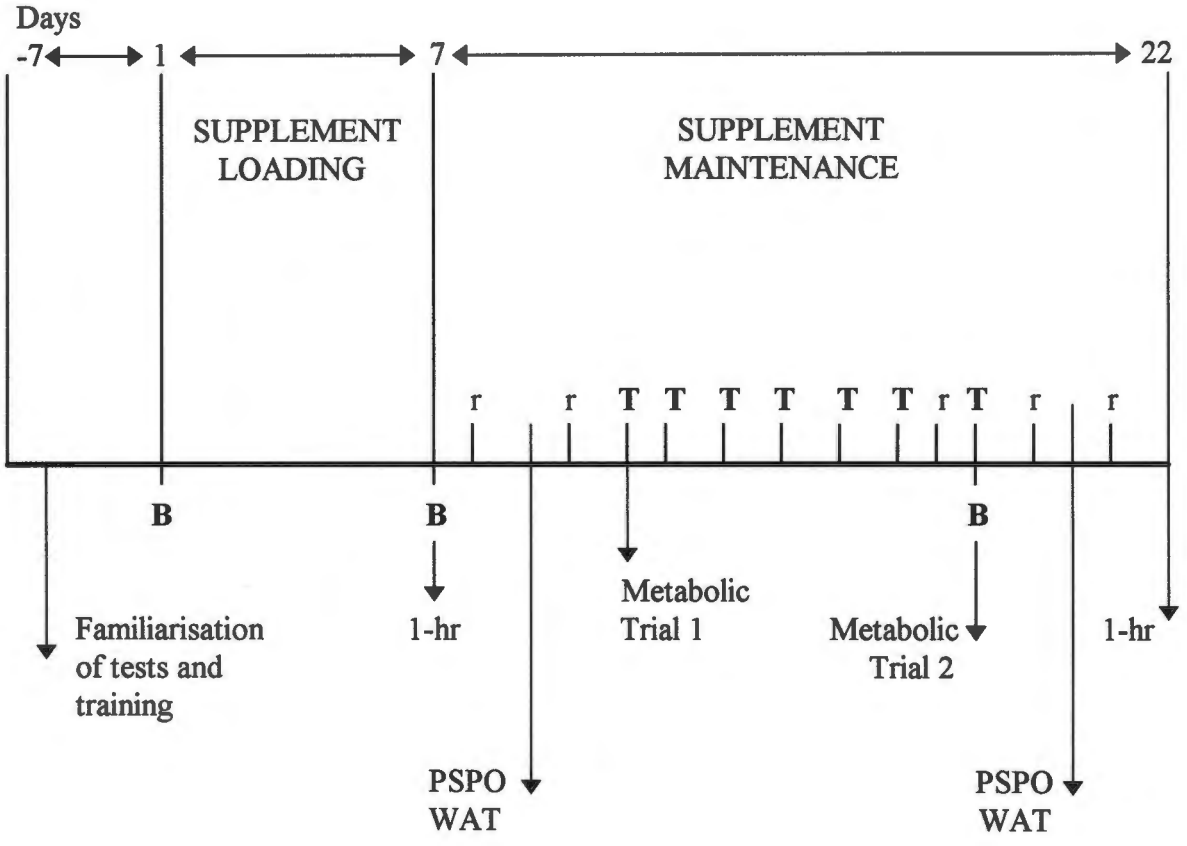

FIGURE 1. Schematic representation of the experimental design. B, biopsy; T, sprint training session; r, rest day; PSPO, peak sustained power output test; WAT, Wingate anaerobic power test; 1-hr, 1-hr distance trial.

An initial muscle biopsy was taken on the first day of the subject's supplementation week (prior to beginning supplementation), and a second biopsy was taken on the 7th day of supplementation. The final biopsy was taken on the 7th day of sprint training before the training bout. This was after a day of rest in order to avoid the influence of any acute effects of the last training session on the muscle sample (Figure 1).

4.1.3 Training Record

Subjects were requested to maintain a consistent level of outdoor endurance training during the experimental trial. They recorded their training on a daily log sheet. In addition, subjects recorded their dietary intake for the three days prior to each biopsy, and were instructed to keep this as consistent as possible.

4.1.4 Supplementation

The creatine monohydrate supplement (Ergomax, AMS Ltd, England) and placebo (glucose tablets that were supplied by AMS Ltd, England, and were shaped identically) were administered in a double-blind fashion. During the loading week, subjects ingested 5 x 1 g colourless and odourless effervescent tablets 4 times per day for 7 days. This approximated a dose of 20 g of creatine per day. The tablets were taken dissolved in warm fluid at the 3 main mealtimes and before the daily training bout (in the event of a "rest day", the 4th dose of tablets was taken immediately before sleep). Times of ingestion and physical and psychological states (and any possible side-effects) were recorded daily on a log sheet.

During the week of sprint training and until the last performance test, subjects ingested a maintenance dose of 2 x 1 g of creatine monohydrate or placebo per day.

4.1.5 Intermittent sprint training

Subjects reported to the laboratory on 6 consecutive days, and then once more following a day of rest for their sprint training. The training was performed on the Wingate Monark ergometer and consisted of a short warm-up (10 min of light cycling), and 10 bouts of 10 s maximal sprint cycling with 140 s active recovery (no resistance) between bouts. The load

on the ergometer was set at 0.1 kilopond/kg body mass. This particular training protocol was chosen so that the work bouts elicited maximal use of the phosphagen and adenine nucleotide energy pathways, and the rest bouts facilitated almost complete resynthesis of high energy phosphates during recovery. It should be noted that the one week of training was not chosen to elicit a training adaptation, but rather to elicit a marked decrease in resting muscle total adenine nucleotide levels as previously shown using a similar protocol (Hellsten-Westing et al., 1993b).

Training took place at the same time every day, and subjects were permitted to drink water *ad libitum*. Heart-rate was monitored during training on each day via a Polar heart-rate monitor (Polar, Helsinki, Finland). Also, a subjective rating of perceived exertion was made by each subject at the end of each training session using a modified Borg scale with ratings from 6 to 20 (Appendix III). Subjects maintained their usual outdoor endurance training during the week.

4.1.6 Exercise performance and metabolic tests

Before and after the week of intermittent sprint training, subjects were required to undergo three performance tests. These included a peak sustained power output test (PSPO), a 1-hr distance trial, and a 30 s Wingate anaerobic test (WAT). The WAT and PSPO test were performed on the same day (PSPO first, WAT second) separated by 30 min of inactive recovery. During both tests, subjects were instructed to remain seated, and they were given strong verbal encouragement. During the 1-hr test, subjects were aware of the time elapsed since the start of the test, but were blinded to the distance covered until after the whole trial was completed. Subjects were permitted to drink water *ad libitum* during the tests. Details of these tests are described in Chapter 3.

In order to examine whether creatine loading prior to, and maintenance during sprint training had any effect on the metabolic response to intermittent sprint exercise, the first and last day of the week of sprint training were used as metabolic tests. Mixed venous blood samples were taken at rest; immediately after the 5th and 10th bout of exercise; and at 5, 10, 20, and 30 min during the recovery from exercise. Details of the blood sampling and analysis methods are described in Chapter 3.

4.1.7 Statistical analysis

The results are expressed as means ± SEM, unless otherwise stated. Analyses were performed using the Statistica Analysis software package (Statsoft Inc., Tulsa, Oklahoma). A Student's t-Test for unpaired samples was employed when comparing the subject characteristics of each group. For the performance and muscle data, a two-way analysis of variance (ANOVA) with repeated measures was used to compare the main effect of group within each trial. The blood data was analysed using a three-way ANOVA with repeated measures, where the group means were compared within each trial and time point. Where significance was indicated a Tukey NSD post-hoc analysis for unequal sample size was performed. The relationship between creatine loading and other factors (such as fibre type and weekly training distance) was examined using a Pearson Product Moment Correlation Coefficient. The level of significance used to reject the null hypothesis was $p < 0.05$.

4.2 Results

After all analyses were completed, the investigators were unblinded. Seven subjects had taken the creatine supplement and 6 subjects had taken the placebo. Subject characteristics are presented in Table 3. The subjects' road endurance cycle training was consistent during the 2 weeks of the testing and laboratory training.

TABLE 3. Subject Characteristics

	Placebo Group	Creatine Group
Age (yr)	24.3 ± 1.5	24.0 ± 2.6
Height (cm)	177.3 ± 0.04	176.8 ± 0.04
Mass pre-supplementation (kg)	72.4 ± 3.6	69.0 ± 4.3
Mass post-supplementation (kg)	73.2 ± 3.7 *	70.1 ± 4.4 *
Mass post-training (kg)	72.8 ± 3.7	70.5 ± 4.4
Road training during supplement loading (km/d)	35.6 ± 4.5	35.0 ± 6.9
Road training during supplement maintenance (km/d)	37.3 ± 7.3	33.2 ± 4.0

Values are means ± SEM. Placebo group, n = 6; Creatine group, n = 7. * Significantly different from pre-supplementation.

4.2.1 Data collected at rest

The muscle samples from 2 subjects were too small for accurate analysis, therefore the results of the muscle substrates and metabolites represent data from 5 subjects in the placebo group and 6 subjects in the creatine group. All muscle biopsies were taken at rest, thus results represent resting concentrations.

a.) Creatine loading

Prior to either placebo or creatine ingestion, the mean total creatine content (TCr) for each group was similar to previously reported levels (Harris et al., 1992; Harris et al., 1974). As shown in Figure 2, the oral creatine supplementation regimen of 20 g creatine monohydrate per day for 7 days significantly ($p < 0.05$) elevated skeletal muscle TCr by an average of 21% (121 ± 4 mmol/kg dm to 147 ± 9 mmol/kg dm), while TCr levels in the placebo group remained unchanged (122 ± 4 mmol/kg dm to 125 ± 4 mmol/kg dm). The upper

limit of muscle creatine content after loading was 177 mmol/kg dm. Of the increase in the TCr pool, approximately 94% resulted from a rise in creatine content alone. When analysed separately, PCr and Cr did not change significantly (PCr for the placebo group pre: 74.1 ± 5.6, post: 70.5 ± 6.2; creatine group pre: 77.8 ± 5.3, post: 77.6 ± 3.7mmol/kg dm) (Cr for the placebo group pre: 47.4 ± 1.9, post: 54.4 ± 6.5; creatine group pre: 45.0 ± 6.4, post: 69.3 ± 6.9 mmol/kg dm).

Within the creatine supplemented group, the increase in TCr varied extensively between subjects (ranging from +8.8 to +54 mmol/kg dm). Three subjects appeared to have very low creatine uptake (< 20mmol/kg dm), and one subject (No. 1) did not increase his TCr content above 120 mmol/kg dm (Figure 3). This variance in the extent of creatine uptake into the muscle was not related to the subjects' initial total creatine content ($r = 0.09$, NS). Also, the change in TCr did not correlate significantly with the subjects' weekly road training distance ($r = -0.37$, NS). In contrast, there was a highly significant correlation between the increase in muscle TCr and the percentage of Type IIB muscle fibres ($r = 0.95$, $p < 0.005$) (Figure 4). This relationship was specific to Type IIB fibres and did not hold for the sum of all Type II fibres ($r = 0.42$, NS). The percentage of Type IIB fibres did not correlate significantly with the initial (pre-supplementation) TCr content ($r = 0.17$, NS, $n = 11$), but did correlate with the final (post-supplementation) TCr content ($r = 0.89$, $p < 0.05$, $n = 6$). Percent fibre type and area of skeletal muscle fibres for each group are presented in Table 4. Creatine uptake did not correlate with either percent Type IIA fibres ($r = -0.42$, NS) nor percent Type I fibres ($r = -0.45$, NS)

TABLE 4. Percent fibre type (%) and area (μm) in skeletal muscle fibres of the placebo and creatine groups.

	Placebo Group	Creatine Group
Percent Type I	43.3 ± 2.2	40.4 ± 3.2
Percent Type IIA	26.3 ± 1.3	30.8 ± 3.1
Percent Type IIB	30.6 ± 3.1	28.7 ± 1.5
Area Type I	5240 ± 315	4781 ± 116
Area Type IIA	5332 ± 606	5331 ± 330
Area Type IIB	5983 ± 665	5445 ± 302

Values are means ± SEM.

During the week of creatine or placebo loading, there was a significant increase in body mass ($p < 0.05$) independent of the group. The mean increase in body mass was similar in both groups (placebo group: 1.2 kg; creatine group: 1.1 kg). However, the subject who showed the largest increase in mass (2.05 kg), also had the largest increase in TCr content (+ 54 mmol/kg dm), although this relationship did not form a trend in all the subjects ($r = -0.16$, NS). Both groups' body mass was relatively unchanged following the 7 days of training (mean change in placebo group: - 0.4 kg; creatine group: + 0.4 kg) (Table 3).

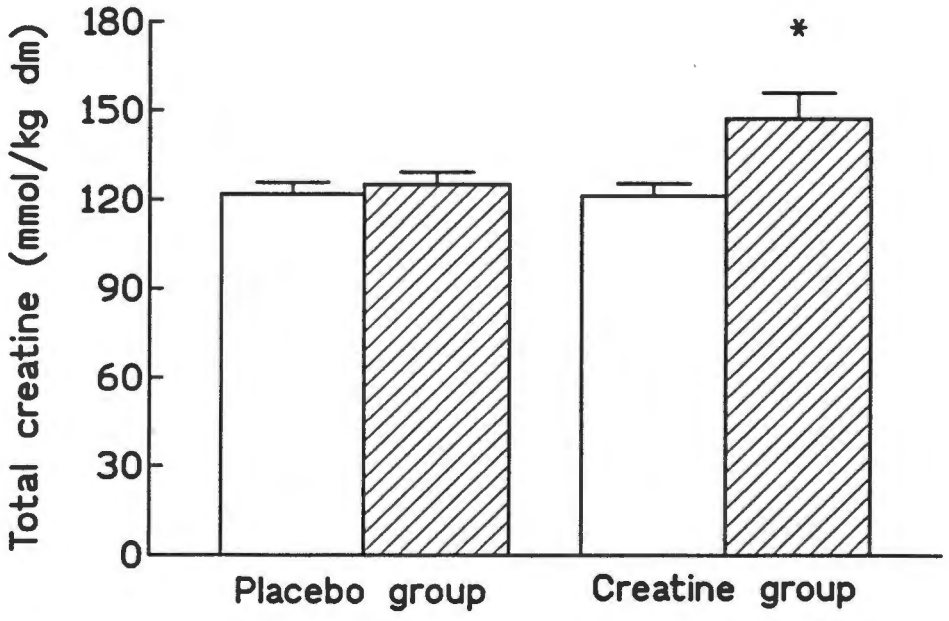

FIGURE 2. Skeletal muscle total creatine content before (□) and after (▨) supplementation with either 20 g of creatine monohydrate or placebo per day for 7 days. Values are means ± SEM. * Significantly different from before supplementation and placebo group ($p < 0.05$).

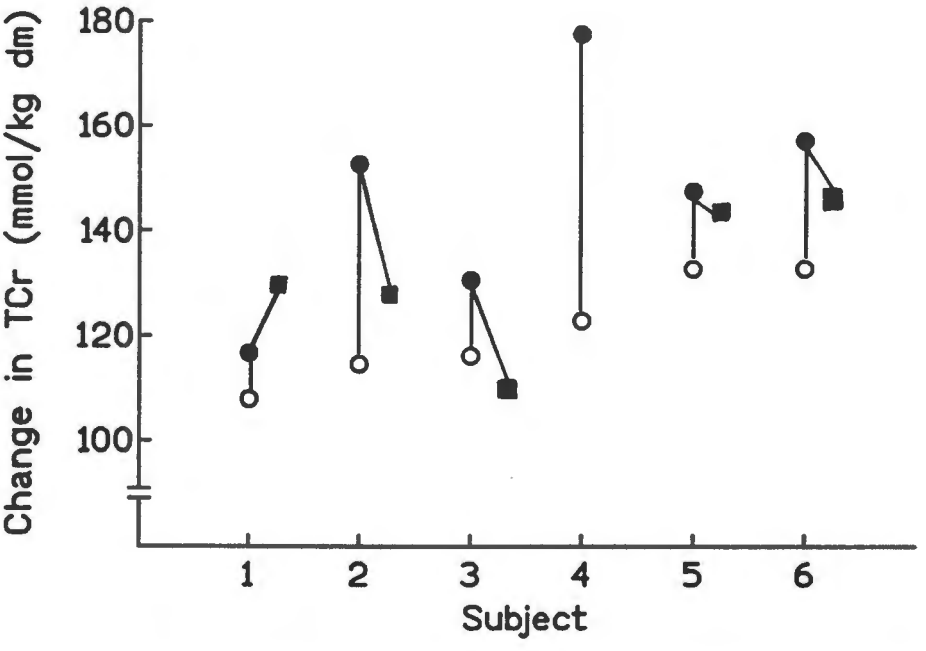

FIGURE 3. Skeletal muscle total creatine content (TCr) before (o) and after (•) supplementation with 20 g creatine monohydrate per day for 7 days, and after a maintenance dose of 2 g per day for 15 days (■) for each subject who had been randomised to the creatine group.

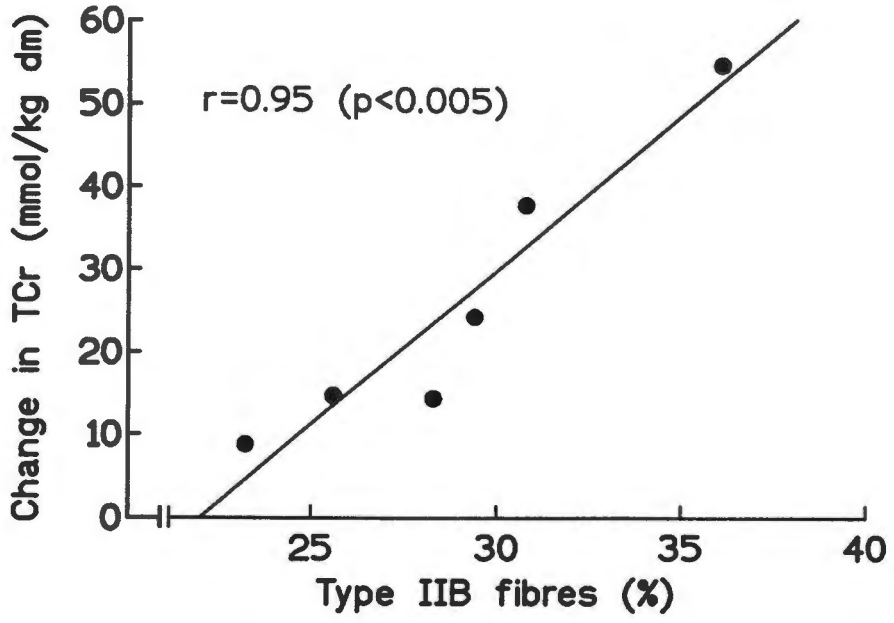

FIGURE 4. The relationship between the increase in skeletal muscle total creatine content (TCr) after supplementation with 20 g of creatine monohydrate per day for 7 days and the percentage of skeletal muscle Type IIB fibres.

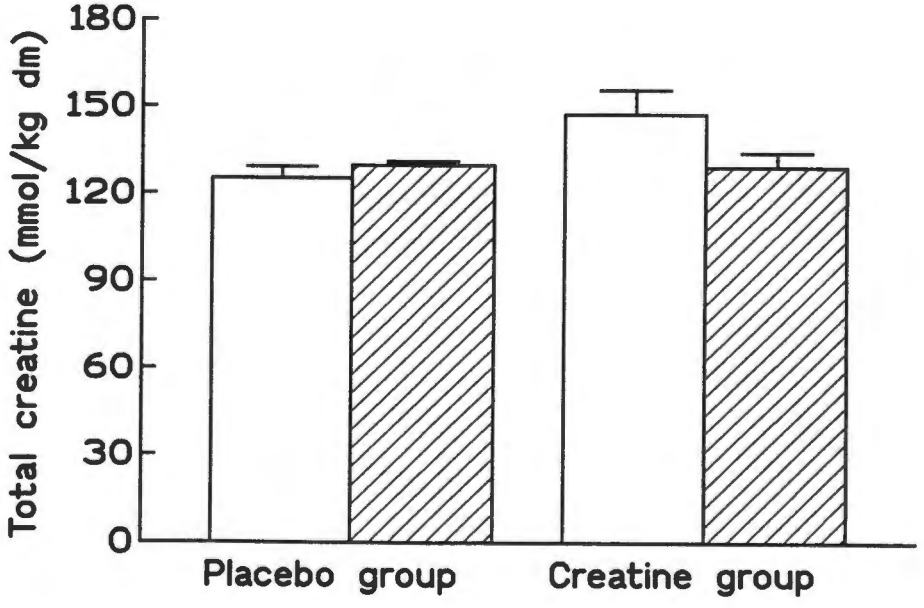

FIGURE 5. Skeletal muscle total creatine content before (□) and after (▨) 6 days of sprint training during which subjects ingested 2 g of either creatine monohydrate or placebo per day. Subjects had ingested a loading dose of 20 g of creatine monohydrate or placebo per day for 7 days prior to sprint training. Values are means ± SEM. No significant difference from before training or between the groups.

As depicted in Figure 5, the creatine-loaded group showed a 12.3% decrease in TCr content from 147 ± 9 mmol/kg dm before the sprint training to 129 ± 5 mmol/kg dm after the training, despite ingesting a maintenance dose of creatine. Although this was a marked decrease, it was not quite statistically significant ($p = 0.08$). Due to this reduction however, the TCr content in the creatine group by the end of the training week was no longer significantly greater than TCr content in the placebo group. Muscle TCr concentrations of the placebo group were similar before and after training (125 ± 4 mmol/kg dm to 130 ± 1 mmol/kg dm).

The week of sprint training did not have a significant effect on muscle PCr levels (creatine group: pre: 77.6 ± 3.7, post: 72.0 ±2.9; placebo group: pre: 70.5 ± 6.2, post: 74.7 ± 6.0 mmol/kg dm).

No ill effects of supplementation were noted.

b.) Adenine nucleotides

As represented in Figure 6 and Figure 7 respectively, the 6 days of sprint training resulted in a significant ($p < 0.05$) reduction in both skeletal muscle ATP content and the total adenine nucleotide pool (TAN = the sum of ATP and ADP) in both the creatine and placebo groups. AMP levels were below the detection limit of the analysis method (< 1 mmol/kg dm), and hence are not reported or included in TAN. Mean resting muscle ATP content fell from 24.1 ± 0.8 to 17.2 ± 0.5 mmol/kg dm in the creatine group, and from 26.5 ± 1.1 to 18.0 ± 0.6 mmol/kg dm in the placebo group after the training. The percentage reduction in ATP content from before training was not different between the two groups (creatine group: 27.2 ± 4 %; placebo group: 30.0 ± 2 %). This finding was similar for the total adenine nucleotide pool which showed a 25.1 ± 4 % reduction in the creatine group (27.3 ± 1.0 to 20.0 ± 0.4 mmol/kg dm), and a 27.6 ± 3 % reduction in the placebo group

(29.8 ± 1.4 to 20.9 ± 0.7 mmol/kg dm). Although the values were marginally lower after the sprint training, resting ADP concentrations were not statistically significantly different before and after the training or between the groups (creatine group pre: 3.2 ± 0.3 vs. post: 2.8 ± 0.2; placebo group: pre: 3.3 ± 0.2 vs. post: 2.9 ± 0.2 mmol/kg dm).

The loss in adenine nucleotides after training was not related to any fibre type composition in particular. More specifically, ATP loss did not correlate with percent Type I ($r = 0.49$, NS), percent Type IIA ($r = -0.62$, NS), or percent Type IIB fibres ($r = 0.66$, NS).

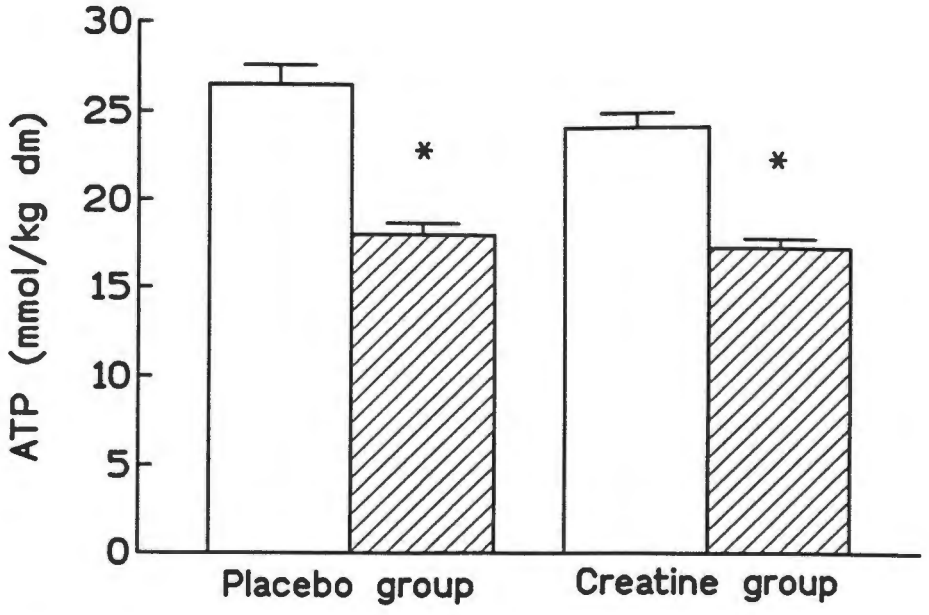

FIGURE 6. Skeletal muscle adenosine 5'-triphosphate (ATP) concentration before (□) and after (▨) 6 days of sprint training during which subjects ingested a maintenance dose of 2 g of either creatine monohydrate or placebo per day. Subjects had ingested a loading dose of 20 g per day for the 7 days prior to sprint training. Values are means ± SEM. * Significantly different from before training ($p < 0.05$).

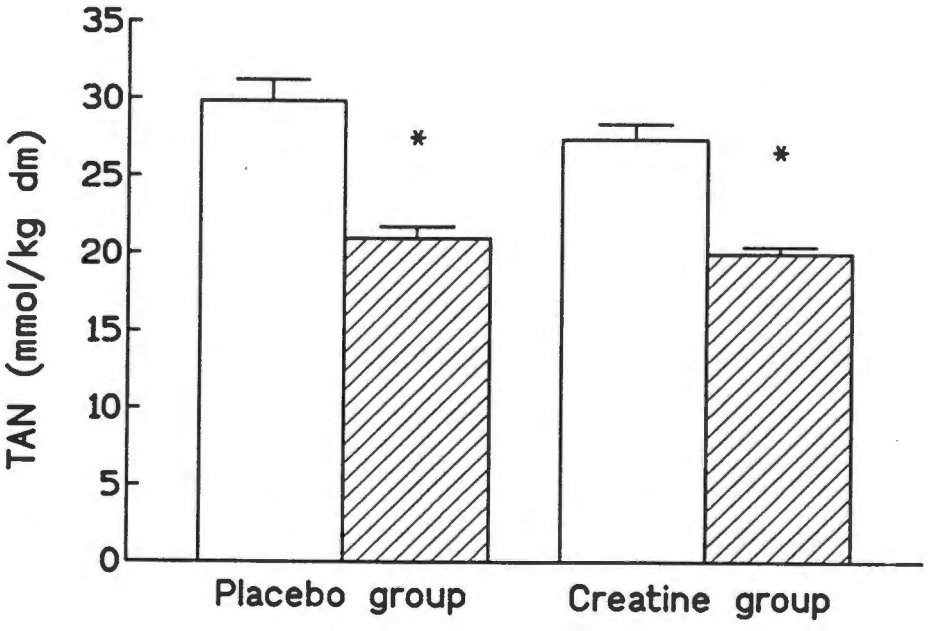

FIGURE 7. Skeletal muscle total adenine nucleotide (TAN = ATP + ADP) concentration before (□) and after (▨) 6 days of sprint training during which subjects ingested a maintenance dose of 2 g of either creatine monohydrate or placebo per day. Subjects had ingested a loading dose of 20 g per day for the 7 days prior to training. Values are means ± SEM. * Significantly different from before training ($p < 0.05$).

4.2.2 Data collected during exercise

a.) Blood data

The week of intermittent sprint training had a pronounced effect on the products of adenine nucleotide degradation measured in plasma samples. These samples were taken during exercise and recovery on days 1 (trial 1) and 7 (trial 2) of the sprint training. Both days were preceded by a rest day.

During the 10 sprint bouts, plasma ammonia (NH_3) concentration increased significantly in both groups, with peak values in excess of 200 μmol/l in some subjects. Ammonia accumulation in trial 2 was significantly ($p < 0.05$) lower than trial 1 for both the creatine and placebo groups (Figure 8, see Appendix VI for numeric values). During the recovery period, the post-training concentrations were on average 27% and 25% lower than the pre-training concentrations for the creatine and placebo groups respectively. In the creatine group the lower plasma NH_3 accumulation in trial 2 was visually evident (see Figure 8) from before the 5th sprint bout (111 ± 12 μmol/l in trial 2, vs. 142 ± 18 μmol/l in trial 1), while the placebo groups' plasma NH_3 levels in trial 2 were only different in the recovery period (i.e. after the 10th sprint bout). This subtle difference between the two groups was not sufficient to meet the level of statistical significance established for this study. Indeed, differences were only significant between trial 1 and 2 at 10 mins in the recovery from the training session for both groups.

The training bouts also resulted in a significant increase in plasma hypoxanthine and urate concentrations which became evident towards the end of the sprint bouts and during recovery from the exercise. As was found for plasma ammonia concentration, both groups displayed significantly ($p < 0.05$) lower plasma hypoxanthine and urate levels after the week of training. (Figure 9 and Figure 10 respectively, see Appendices VII and VIII for

numeric values). The decreased plasma accumulation of hypoxanthine was evident as early as the 5th sprint bout and remained lower throughout the recovery period. The training resulted in an average reduction in hypoxanthine levels that was similar between groups (creatine group ~ 2.4-fold lower; placebo group ~ 2.3-fold lower). Although the creatine group exhibited mean plasma hypoxanthine values that were up to 18% lower than that of the placebo group at any given time point both pre- and post-training, the individual variation rendered this difference statistically insignificant.

The training-induced difference in plasma urate concentration was more delayed than that of either NH_3 or hypoxanthine, and only became conspicuous at 10 mins in the recovery from exercise. The groups showed a comparable decrease in the extent of urate accumulation following training: by 30 mins of recovery from exercise, urate concentrations were on average 80% and 84% of the mean pre-training values at the same time point for the creatine and placebo groups respectively.

Plasma lactate concentration increased significantly during the 10 sprint bouts.
In contrast to the products of adenine nucleotide degradation, lactate accumulation was very similar between the two trials and the two groups. Also, there was less individual variance between the subjects. As can be seen in Figure 11, plasma lactate concentration peaked approximately 5 mins post-exercise with values of up to 18.3 ± 1.1 mmol/l.

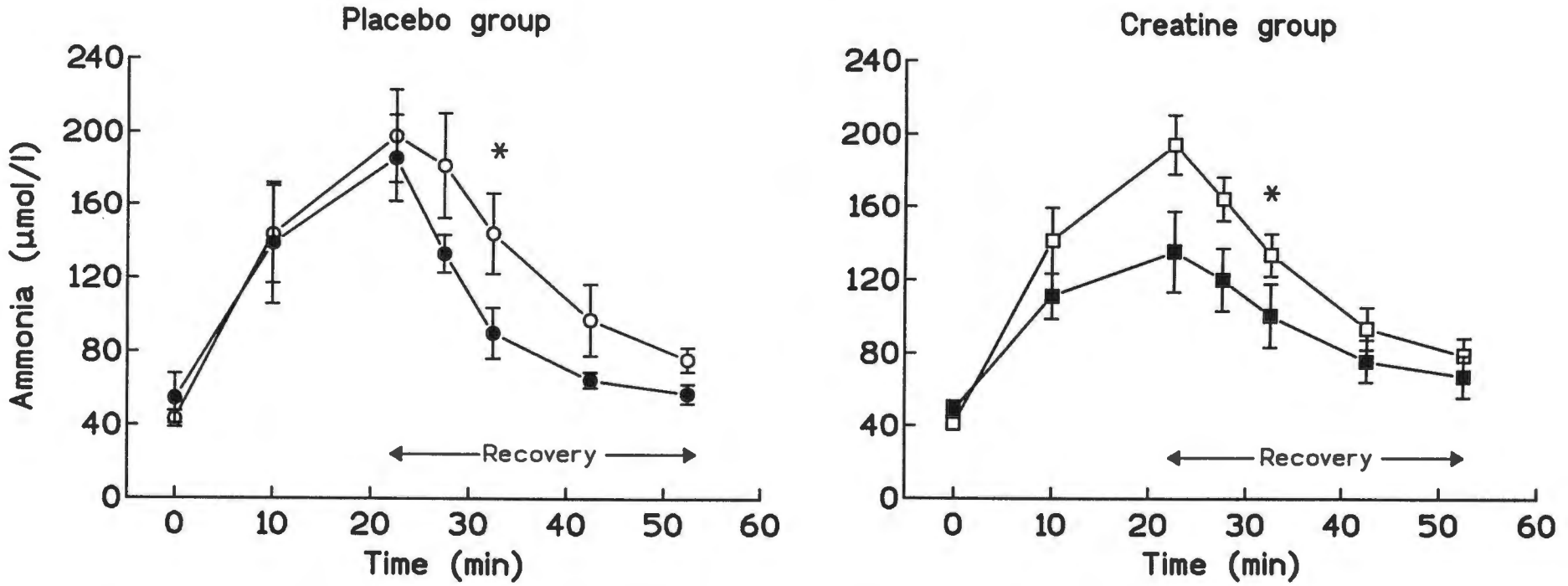

FIGURE 8. Plasma ammonia concentration measured at rest, during, and for 30 minutes after 10 x 10 s sprint bouts with 2 min 20 s active recovery, on the 1st (o, □) and 7th (•, ■) day of such training during which subjetcs ingested a maintenance dose of 2 g of either creatine monohydrate (n = 7) or placebo (n = 6) per day. Subjects had ingested a loading dose of 20 g for the 7 days prior to sprint training. Values are means ± SEM. * Significant difference between 1st and 7th day of training ($p < 0.05$).

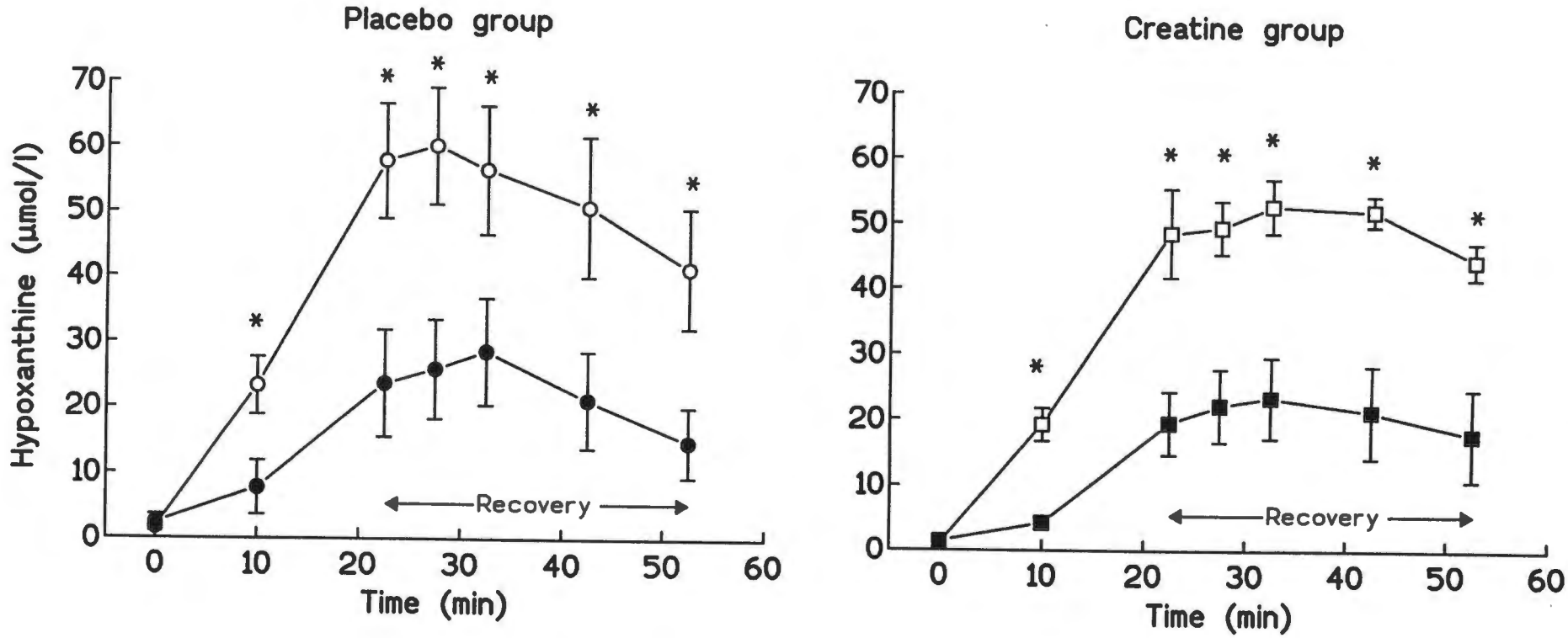

FIGURE 9. Plasma hypoxanthine concentration measured at rest, during, and for 30 minutes after 10 x 10 s sprint bouts with 2 min 20 s active recovery, on the 1st (o, □) and 7th (•, ■) day of such training during which subjects ingested a mainenance dose of 2 g of either creatine monohydrate (n = 7) or placebo (n = 6) per day. Subjects had ingested a loading of 20 g per day for the 7 days prior to training. Values are means ± SEM. * Significant difference between 1st and 7th day of training ($p < 0.05$).

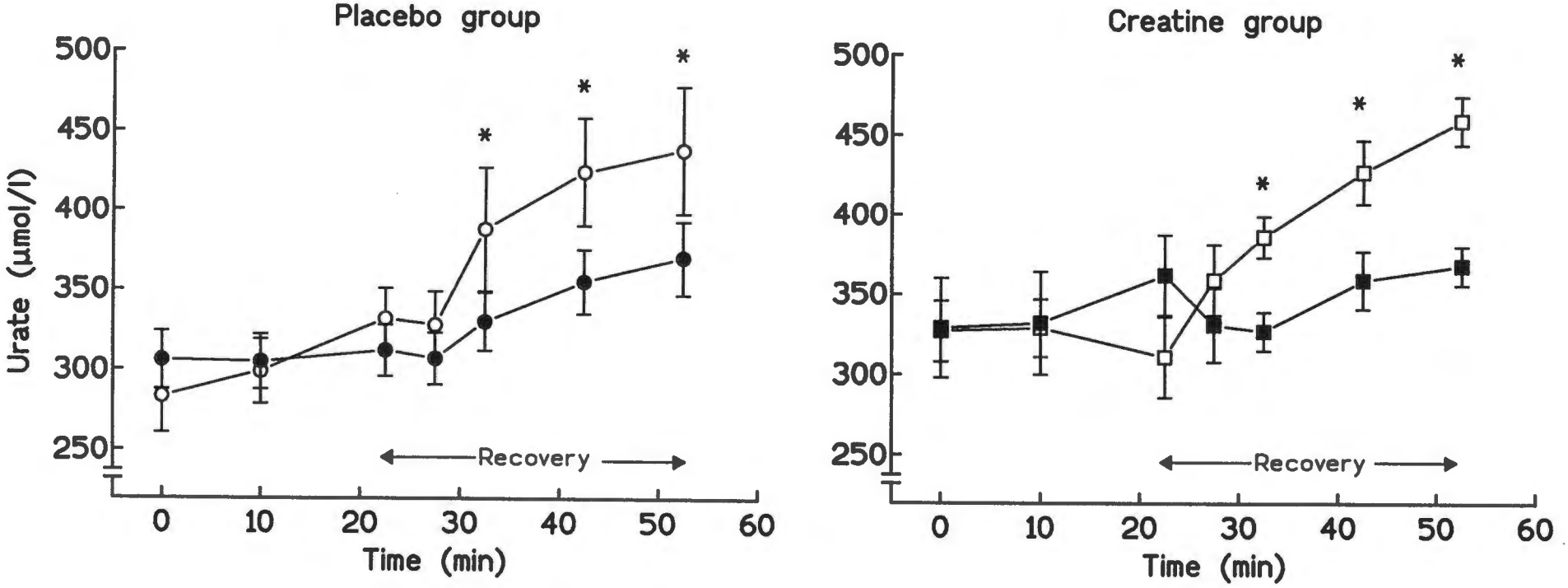

FIGURE 10. Plasma urate concentration measured at rest, during, and for 30 minutes after 10 x 10 s sprint bouts with 2 min 20 s active recovery, on the 1st (o, □) and 7th (•, ■) day of such training during which subjects had ingested a maintenance dose of 2 g of either creatine monohydrate (n = 7) or placebo (n = 6) per day. Subjects had ingested a loading dose of 20 g per day for the 7 days prior to training. Values are means ± SEM. * Significant difference between 1st and 7th day of training ($p < 0.05$).

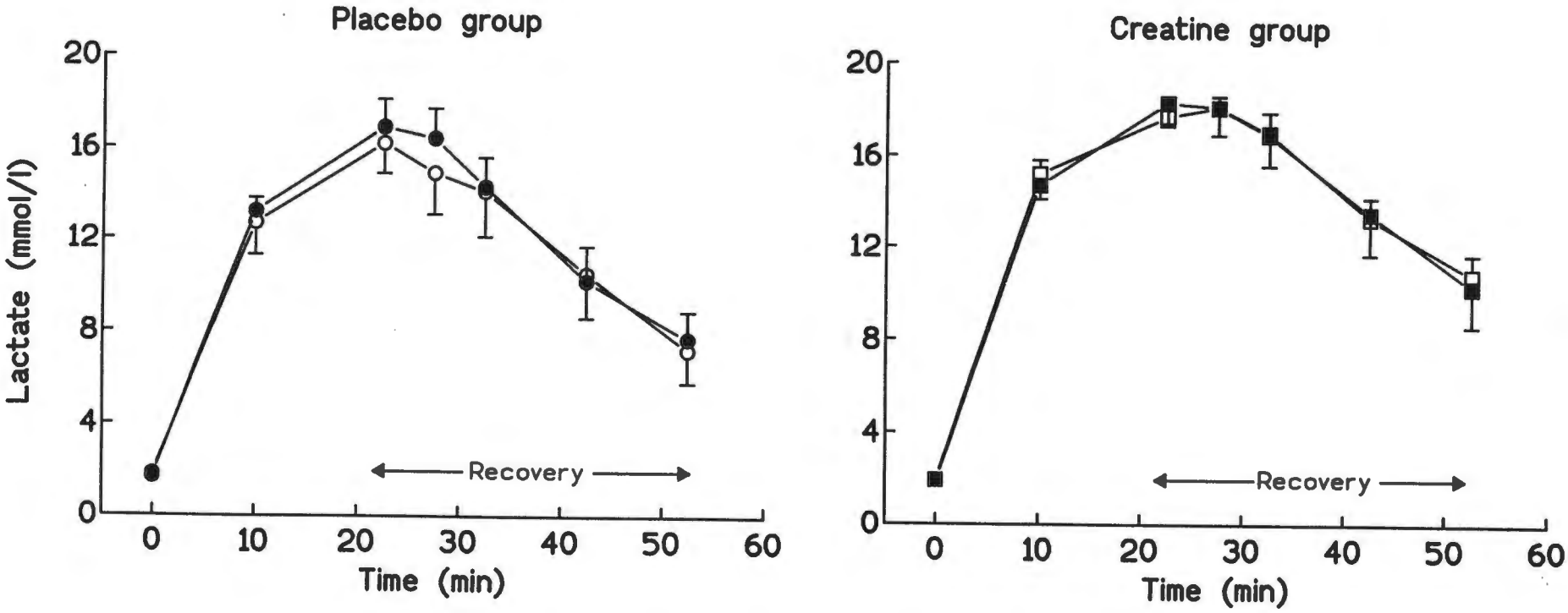

FIGURE 11. Plasma lactate concentration measured at rest, during, and for 30 minutes after 10 x 10 s sprint bouts with 2 min 20 s active recovery, on the 1st (o, □) and 7th (•, ■) day of such training during which subjects had ingested a maintenance dose of 2 g of either creatine monohydrate (n = 7) or placebo (n = 6) per day. Subjects had ingested a loading dose of 20 g per day for the 7 days prior to training. Values are means ± SEM. No significant difference between 1st and 7th day of training ($p < 0.05$).

b.) Performance data

The results of the performance tests are presented in Table 5.

Peak sustained power output (PSPO) was significantly ($p < 0.05$) higher in the creatine group after training compared to the placebo group who remained comparatively unchanged. This change in PSPO in the creatine group represents only a 2.23% increase while PSPO decreased in the placebo group by 1.36%. The distance covered in the 1-hour cycle test did not change significantly after the training and did not differ between the two groups.

In the 30 s Wingate test, peak power; mean power; and peak cadence were all significantly increased after the training ($p < 0.05$, $p < 0.01$, $p < 0.05$ respectively). This improvement was similar in both groups. The 12% and 10% improvement in peak power of the placebo and creatine groups respectively may be almost completely accounted for in the increased peak pedal frequency (12% and 7% respectively). The placebo groups' fatigue rate increased from trial 1 to trial 2 by 37% (from 20 ± 1.9 to 27 ± 3.6 W/s) in contrast to an increase of only 8% (from 19 ± 1.2 to 21 ± 3.1 W/s) in the creatine group. However, this difference was not significant ($p = 0.19$).

c.) Heart-rate and subjective evaluations

In both groups, the heart-rate response during trial 1 and trial 2 was comparable before and after training (Figure 12). On average, subjects were exercising at up to 88.3% (creatine group) and 88.7% (placebo group) of their age-predicted maximum heart-rate during the sprint bouts.

The subjective ratings of perceived exertion acquired at the end of each training session were not significantly different between the creatine and placebo groups during the training. Both groups found the exercise slightly easier towards the 5th day and ratings fell from 15 ± 1 units on the 1st day to 13 ± 1.4 units on the 7th day, and from 14.6 ± 0.7 to 13.7 ± 0.6 units, for the placebo and creatine groups respectively over the whole week's training. This decrease was not statistically significant.

Four of the seven subjects who ingested the creatine supplement (57%) made a special note in their logbooks that they felt "strong" or "good" during the later days for the training, while only one subject who ingested the placebo (17%) made such a comment.

TABLE 5. Performance in a variety of exercise tests before and after 7 days of intermittent training (10 x 10 s sprints with 2 min 20 s active recovery) during which subjects ingested a maintenance dose of 2 g of either creatine monohydrate or placebo per day. Subjects had ingested a loading dose of 20 g per day for the 7 days prior to sprint training.

	Placebo Group		Creatine Group	
	Pre-training	**Post-training**	**Pre-training**	**Post-training**
Peak Sustained Power Output (W)	357.2 ± 18.4	352.3 ± 20.5	349.9 ± 22.6	358.0 ± 23.9 †
1-hr Distance (km)	37.5 ± 0.9	38.6 ± 1.0	39.2 ± 1.0	39.5 ± 1.0
30s Peak Power (W)	1138.2 ± 80.2	1272.8 ± 73.4*	1051.9 ± 52.3	1154.7 ± 56.3*
30s Peak Cadence (rpm)	174 ± 11.6	194.8 ± 10.2*	169.9 ± 7.0	182.1 ± 11.6*
30s Mean Power (W)	692.2 ± 24.2	732.0 ± 12.8*	719.3 ± 35.0	768.9 ± 37.3*
30s Fatigue Rate (W/s)	20.0 ± 1.87	27.4 ± 3.64	19.1 ± 1.2	20.7 ± 3.1

Values are means ± SEM. Placebo group, n = 6; Creatine group, n = 7. W, watts; W/s, watts per second; rpm, revolutions per minute; km, kilometers. † Significantly different from the placebo group and pre-training ($p < 0.05$). * Significantly different from pre-training ($p < 0.05$).

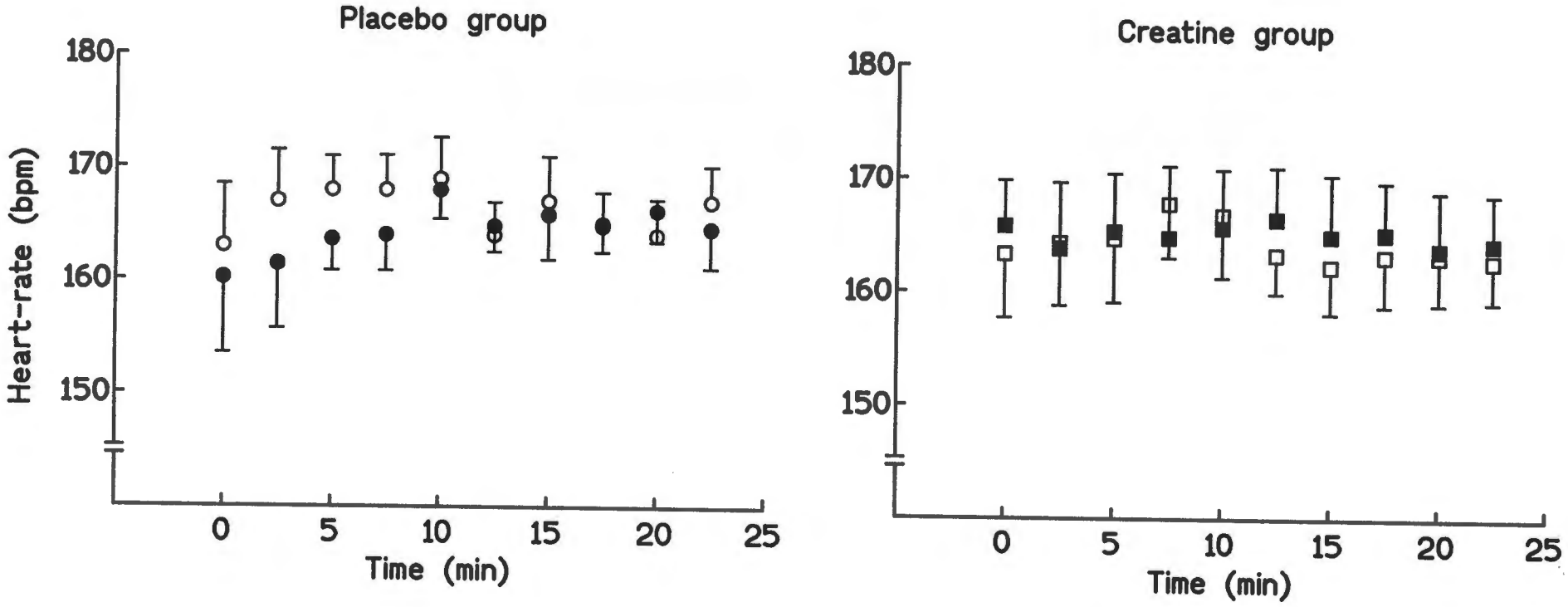

FIGURE 12. Peak heart-rate after each of 10 x 10 s sprint bouts with 2 min 20 s active recovery, on the 1st (o, □) and 7th (•, ■) day of such training during which subjects ingested a maintenance dose of 2 g of either creatine monohydrate (n = 7) or placebo (n = 6) per day. Subjects had ingested a loading dose of 20 g per day for the 7 days prior to training. Values are means ± SEM. * Significant difference between 1st and 7th day of training ($p < 0.05$).

4.3 DISCUSSION

The major findings of the present study which will be discussed below are that 1) the uptake of creatine into the muscle following oral creatine supplementation is related to the percentage of Type IIB muscle fibres; 2) muscle total creatine (TCr) content after supplement loading is not maintained during intermittent sprint training despite a maintenance dose of 2 g per day; 3) intermittent sprint training improves sprint performance, and has a significant effect on adenine nucleotide metabolism.

4.3.1 Creatine loading

In this study, oral creatine supplementation markedly increased creatine uptake into skeletal muscle, but the extent of this ranged from 8 mmol/kg dm to 54 mmol/kg dm. When interpreting these results, the possible effects of low subject numbers and relatively small changes in TCr in 50% of the subjetcs ought to be borne in mind. As far as the authors are aware, this is the first study to show that the extent of increase in TCr after creatine supplementation is linearly and positively related to the pecentage Type IIB muscle fibres. Since the individual variation in fibre type composition is considerable (in the present study the percentage of Type IIB fibres ranged from 19% to 40%), this may also be an explanation for the previous findings of large variations in the individual responses to a similar creatine supplementation regimen (Greenhaff et al., 1994a; Harris et al., 1992; Lemon et al., 1995A).

There are a few possible reasons that may explain this phenomenon. The functions of PCr as an energy reservoir for the rapid resynthesis of ATP, and as a shuttle for high energy phosphates from the mitochondria to the myofibril, make PCr and creatine integrally related to adenine nucleotide (AN) metabolism. Considering that the regulation of AN metabolism varies between the fibre types (Dudley and Terjung, 1985a; Meyer et al., 1980;

Meyer and Terjung, 1979; Tullson et al., 1990), it is likely that creatine and PCr levels are also regulated differently in different fibre types. In addition, it is probable that differences in AN metabolism are evident not only between Type I and Type II fibres in general, but more specifically between Type IIA and IIB fibres. Such differences have been shown between fast glycolytic and fast oxidative-glycolytic fibres in rats (Dudley and Terjung, 1985a). In particular, fast glycolytic fibres displayed significantly greater ATP and PCr reduction and greater AMP deaminase activity, than fast oxidative-glycolytic fibres, in response to an electrical stimulus (5 Hz) in *in situ* rat muscle. As these differences were eliminated when blood flow was occluded, the authors concluded that differences between the fibres occured relative to the muscle fibre's functional capacity for aerobic metabolism.

Thus, variance in the regulation of AN metabolism appears to be consistent with the metabolic profiles of Type I and Type IIA, and Type IIB fibres. Given that Type IIB fibres are maximally recruited only during very high-intensity exercise (Burke and Edgerton, 1975; Vollestad et al., 1984) in which the acceleration of energy production is fast, it is not surprising that 1) resting PCr content in Type II muscle fibres is significantly greater than in Type I muscle fibres (Edstrom et al., 1982; Greenhaff et al., 1994A; Tesch et al., 1989), and 2) the extent of PCr degradation and utilisation rates are greater in Type II fibres during a 30 s treadmill sprint (Greenhaff et al., 1994A). A higher muscle TCr content would make Type IIB fibres more suited to respond to maximal energy demands. Thus, the preferential increase in TCr in Type IIB fibres after supplementation may be a reflection of their greater capacity to accommodate a higher TCr content. A significant relationship was not found between the increase in TCr and either percentage Type IIA fibres, or the sum of the pecentage Type IIA and IIB fibres. These data suggest that the greater PCr content in Type II fibres found previously (Edstrom et al., 1982; Tesch et al., 1989; Greenhaff et al., 1994A) was probably predominanatly due to higher PCr content in Type IIB fibres.

Although the percentage of Type IIB fibres correlated significantly with the *change* in TCr and the final (post-supplementation) TCr contents, it did not correlate with initial (pre-supplementation) TCr levels. This suggests that the characteristic feature of Type IIB fibres that facilitates increases in TCr content with supplementation is likely to be predominantly related to a process of creatine uptake into the muscle fibre under conditions of high plasma creatine concentrations which is not evident under normal conditions, and to a lesser extent, the storage capacity of the muscle fibre.

Other factors have been reported to be related to the variance in the extent of creatine "loading". Greenhaff et al. (1994a) and Harris et al. (1992) found that the extent of creatine loading was dependent on an individual's initial creatine content prior to supplementation. Those subjects with an initial TCr content of close to or less than 120 mmol/kg dm showed the greatest increases in TCr with supplementation. Such a relationship was not evident in this study, although the subject numbers are small. Another variable identified as influencing the extent of creatine uptake into the muscle is exercise. Harris et al. (1992) showed that after supplementation the increase in TCr in 5 subjects who performed 1 hour of hard one-legged exercise per day was significantly greater in the exercised than the sedentary leg. In the present study, despite all subjects performing a substantial amount of road training (>230 km/week), not all subjects loaded substantially with creatine. The extent to which subjects loaded was not related to the amount of road training they performed per week ($r = -0.65$, NS).

In this study the mean increase in muscle TCr of 21% is in agreement with the 20%, 19% and 15% increases reported by Harris et al. (1992), Greenhaff et al. (1994b) and Greenhaff et al. (1994a) respectively after subjects followed a similar supplementation regimen. The maximum TCr content following loading was 177 mmol/kg dm. The high value was not due to a higher normal range for our laboratory, since the lowest TCr measured was 108 mmol/kg dm. This result is comparable to the previously reported level of 183 mmol/kg

dm in one subject who augmented his initial increase in TCr by one-legged hard exercise (Harris et al., 1992). It appears that the physiological upper limit to creatine loading is higher than the previously suggested range of 150 to 160 mmol/kg dm (Greenhaff et al., 1994a; Harris et al., 1992) regardless of the the influence of exercise. In addition, this maximum value occurred in the subject with the highest percentage of Type IIB fibres. The observation that 94% of the increase in TCr was in the form of creatine was notably greater than the ~ 80% found by Harris et al. (1992). Although these data are difficult to understand, it is possible that the lack of equilibrium between creatine and phosphocreatine is a function of the low subject response to creatine ingestion. Alternatively, this may be related to the state of relaxation of each subject prior to or during the muscle biopsy procedure. Isometric contraction of the quadriceps muscle prior to, or on insertion of the biopsy needle into the muscle, may have resulted in a decrease in the relative ratio of PCr : Cr in the muscle, and consequently the concentration of creatine in the muscle would have been higher than that in a truly rested state.

In contrast with other studies (Balsom et al., 1993a; Greenhaff et al., 1994a; Lemon et al., 1995A; Soderland et al., 1994A; Stroud et al., 1994; Viru et al., 1993) the change in body mass in the creatine supplemented group (mean + 1.1 kg) was not statistically significantly different from the placebo group. This is probably due to a comparable gain in mass found in the placebo group (mean + 1.2 kg), and the variance in the response to creatine supplementation. The mechanism by which an increase in body mass accompanies creatine loading is not clear. Two theories proposed suggest that with increased TCr concentration there is 1) an increase in total body water content; and 2) an increase in contractile protein synthesis (Balsom et al., 1993a). Although the latter theory has indirect experimental support (Ingwall et al., 1972; Ingwall et al., 1974; Meyer et al., 1986; Sipila et al., 1981;), other data is not supportive (Fry and Morales, 1980). Although the time of day subjects were tested was controlled, it is proposed that the level of hydration in regularly exercising

athletes may not be consistent and thus may confound the results of studies in which small, consistent gains in body mass from an experimental intervention are expected.

It is apparent from this and other studies that creatine supplementation does not guarantee that muscle TCr stores will increase significantly in all individuals. The effect of creatine supplementation on exercise performance has been widely studied in recent years, but conflicting results have placed its ergogenic effect in some doubt. One possible reason for these conflicting results could be the varying degree to which individuals load with creatine. This issue has not been sufficiently examined previously as many of these studies did not quantify skeletal muscle TCr before and after supplementation. It is suggested that any ergogenic effect of creatine supplementation depends on the extent of this loading which in turn is highly associated with an individual's percentage of fast glycolytic muscle fibres. It is important that factors which influence creatine uptake into the muscle are taken into consideration before creatine is administered to athletes at random.

4.3.2 Creatine maintenance

Following a loading regimen of creatine supplement, it has been suggested that a maintenance dose of 2 g of creatine per day should be sufficient to maintain muscle TCr at a high, "loaded" level (Greenhaff, 1995). Although this particular dosage has not been experimentally tested, it is based on the daily turnover rate of creatine for an individual with an average total muscle creatine content of 120 to 130 mmol/kg dm. (Harris et al., 1974; Harris et al., 1992). In the present study, however, the 2 g per day maintenance dose for 11 days failed to keep TCr significantly elevated, since after the maintenance period, the creatine group no longer had a significantly greater TCr content than the placebo group. This may be due to the unusually high stress that was placed on the high-energy phosphate system during the repeated days of high-intensity training. Also, Crim et al. (1976) proposed that creatinine excretion does not necessarily match creatine synthesis,

but rather creatinine is excreted at a constant fraction (~ 0.016) of the body creatine pool. It is possible that if the total body creatine pool increases with creatine loading, the fraction of this pool excreted as creatinine will also increase. In this study, a theoretical calculation of the amount of the total creatine pool that will be excreted per day (Appendix XI) shows that the placebo group is likely to excrete 2.3 g, while the creatine group is likely to excrete 2.9 g. In the placebo group the 2.3 g is higher than the 2 g previously described due to the assumption that trained athletes in this study have a greater muscle mass than an average 70 kg male. The lack of change in resting TCr content in the placebo group with training, implies that daily turnover of the creatine pool was maintained sufficiently by dietary intake and endogenous synthesis of creatine. However, in the creatine group, the increased TCr pool size resulted in an increase in its turnover of at most 1 g per day. The maintenance dose of 2 g per day ought to have been sufficient to sustain this increased turnover, but this was not the case, and TCr in the creatine group decreased with training. Thus, the excessive sprint training appeared to result in a "draining" of muscle creatine in the loaded, but not the unloaded state. This suggests that the minimum or normal average level of muscle TCr is more tightly regulated than an overloaded level.

It is possible, that measurement of creatinine excretion during and following creatine loading may indicate that the currently advised maintenance dose of creatine may need to be re-evaluated.

4.3.3 Intermittent sprint exercise and training

An acute decrease in intramuscular ATP (Cheetham et al., 1986; Gaitanos et al., 1993; Tullson et al., 1995) and TAN concentration (Sahlin and Broberg, 1990; Tullson et al. 1995) has been commonly observed at completion of a bout of high-intensity exercise. During high-intensity intermittent exercise where the rate of ATP hydrolysis exceeds resynthesis, the accumulating ADP is converted to ATP and AMP. Subsequently, AMP is

deaminated to IMP with the release of ammonia (NH_3) to the plasma. Further degradation of IMP results in the loss of purines from the muscle cell as indicated by plasma accumulation of hypoxanthine and urate. In this study, the high plasma accumulation of NH_3, hypoxanthine and urate during and after the high-intensity intermittent training bouts verified that there was indeed an imbalance between ATP hydrolysis and resynthesis rates and consequently AN degradation. High plasma concentrations of ammonia, hypoxanthine and urate have been previously demonstrated after bouts of sprint exercise (Greenhaff et al., 1994b; Hellsten-Westing, 1993a).

The week of intermittent sprint training elicited a significant decrease in resting TAN and ATP stores in the muscle. The change in ATP and TAN concentrations were not due to an acute exercise response as the biopsy sample was taken 48 hours after the last training session. The reduction of between 28% and 32% was slightly greater, but comparable to that (18% to 23%) described previously (Green et al., 1987; Hellsten-Westing et al., 1993b; Stathis et al., 1994).

Since TAN and ATP contents were still reduced 2 days after the sprint training ended, it would appear that the consecutive days of training resulted in a sustained environment of enhanced IMP catabolism in which the repeated loss of purines from the muscle to the plasma was in excess of purine salvage and *de novo* synthesis. This would be particularly evident in Type II muscle fibres which exhibit a greater degree of AN degradation (Dudley and Terjung, 1985a; Meyer et al., 1980; Meyer and Terjung, 1979; Tullson et al., 1990) and slower rates of *de novo* ATP synthesis than Type I fibres (Tullson et al., 1988), although we did not measure this. It has also been proposed that high-intensity training results in a "down-regulation" in muscle adenine nucleotide content (Hellsten-Westing et al., 1993b; Stathis et al., 1994) which may result in an earlier acceleration of ATP resynthesis or an amplification of the signal due to the comparatively lower concentration of ATP at the onset of exercise.

The present study and previous reports (Green et al., 1987; Hellsten-Westing et al., 1993b; Stathis et al., 1994) of decreased levels of adenine nucelotides are in conflict with other studies which have suggested that high-intensity training 1) does not cause any change in resting muscle AN levels (Boobis et al., 1983A; Nevill et al., 1989; Thorstensson et al., 1975) or 2) causes an increase in AN levels (Eriksson et al., 1975; Houston and Thomson, 1977). These conflicting results may be best explained by differences in the training protocols used in each study. Those studies that showed a decrease in AN content following training used training protocols that were extremely strenuous and consisted exclusively of repetitions of one exercise type at maximal intensity. This implies that there may be a threshold intensity of metabolic stress above which significant AN loss is likely to occur. From this study it is evident that significant AN degradation will occur after consecutive days of intermittent sprint exercise at an intensity that results in a peak plasma lactate level of about 18 mmol/l. Similarly, such a threshold was observed in a study on horses in which significant AN degradation *only* occurred above a blood lactate concentration of 15 mmol/l (Sewell and Harris, 1992). These authors proposed that there was a critical pH below which there may have been a substantial decrease in ADP rephosphorylation by PCr which resulted in a marked increase in ADP degradation. Alternatively it may be related to the marked increase in AMP deaminase activity which is evident at and below a muscle pH of 6.6 units (Dudley and Terjung, 1985b). This is also indirect support for the theory that AN loss is a result of a compromised capacity to replete ATP and thus AN.

An hypothesis of this study was that increased muscle TCr content could decrease this effect by improving ADP rephosphorylation kinetics. However, this study failed to show that increased muscle TCr content attenuates a decrease in ATP and TAN content. This failure may be related to the fact that by the end of the training week the creatine group no longer had a significantly greater TCr content compared to the placebo group. Thus, it is

possible that if the TCr content in the creatine group had remained significantly greater than that in the placebo group, the extent of AN loss with training may have been different between the two groups. This question therefore remains unanswered.

Plasma accumulation of metabolites measured in this study during and after the intermittent sprint bouts were comparable to previously reported levels for ammonia (Greenhaff et al., 1994b); hypoxanthine (Bangsbo et al., 1992; Hellsten-Westing et al., 1993a); urate (Hellsten-Westing et al., 1993a); and lactate (Greenhaff et al., 1994; Withers et al., 1986).

The intermittent sprint training resulted in a decreased accumulation of AN degradation products in the plasma (ammonia, hypoxanthine and urate) during and in the recovery from exercise when comparing the first and last training session. This finding is supported by previous work (Hellsten-Westing et al., 1993a; Snow et al., 1992; Stathis et al., 1994). A lower concentration of ammonia, hypoxanthine and urate in the plasma may be due to one, or a combination of the following: 1) a decrease in the production of that compound in the muscle; 2) a decrease in the efflux of that compound from the muscle (which may relate to increased salvage or reuse of the compound in the muscle); and 3) an increase in the removal of that compound from the plasma.

A mechanism for the decreased accumulation of these compounds cannot be deduced directly from the measurements made in this study. However, the observation that there was a greater training effect on plasma hypoxanthine and urate concentrations than on plasma ammonia concentration suggests that the most pronounced effect occurred after AMP deamination in the AN catabolic pathway. Two means by which plasma hypoxanthine accumulation may be reduced after AMP deamination are firstly, that there is increased IMP reamination via the purine nucleotide cycle (PNC) after training (although this is unlikely as the activity of the reamination limb of the PNC is very low *during* intense exercise) (Meyer and Terjung, 1980; Tullson and Terjung, 1991); and secondly, that there

is increased muscular hypoxanthine salvage to IMP. The latter theory is indirectly supported by Hellsten-Westing et al. (1993a) who reported an increase in the activity of the enzyme responsible for this conversion (hypoxanthine phosphoribosyl transferase, HPRT) after 6 weeks of high-intensity training. Also, after training, an improvement in the clearance of AN degradation products from the plasma may have been greater for hypoxanthine and urate in comparison to ammonia.

Alternatively, lower plasma accumulation of the compounds may be a result of decreased AN degradation due to training. Stathis et al. (1994) reported that the magnitude of decrease in ATP during a sprint bout was reduced by 52% after sprint training despite subjects having a comparatively lower resting ATP concentration after the training. This, and the present study's findings may be related to a decrease in AMP deaminase activity which has been shown to occur after high-intensity training (Hellsten-Westing, 1993a; Tullson and Terjung, 1991), and may be the mechanism for the "down-regulation" previously discussed.

Considering that the half-life of protein is approximately 10 days, it is unlikely that the 7 days of training would provide sufficient time to increase enzyme content or activity significantly. It is possible that the metabolic alteration evident after the training may rather be due to a reorganisation of the localisation of enzymes. This may manifest in an increased efficiency of the PCr-Cr shuttle, as it relies on the compartmentalisation of compounds and enzymes.

Another possibility is that sprint training increases the capacity of oxygen-independent glycolysis at maximal workloads, as has been previously proposed by Nevill et al. (1989) and Linossier et al. (1993). This would imply that the contribution of oxygen-independent glycolysis to energy production is greater at maximal workloads, thus partially relieving the stress on AN metabolism, and attenuating the loss of purines to the plasma. Higher plasma

concentrations of lactate, and increased activity of PFK and LDH after sprint training have been reported as evidence for greater energy production from oxygen-independent glycolysis (Linossier et al., 1993). In contrast however, in this study plasma lactate concentration during the metabolic tests before and after training was similar, and thus the contribution of oxygen-independent glycolysis to energy production appeared to be unaltered by the 7 days of training. Considering that increases in the activities of glycolytic enzymes such as PFK and LDH have as yet only been demonstrated after more than 5 weeks of training (Linnossier et al., 1993; Roberts et al, 1982), it is possible that an adaptation in this system may take longer than 7 days to become evident.

A limitation of this study was that the exact amount of work done during each metabolic test (i.e. day 1 or day 7 of the sprint training) was not quantified. Based on the similarity of plasma lactate data and heart-rate data between day 1 and day 7, it is highly unlikely that the amount of work performed on the 7th day was less than that on the 1st day of training. Considering this, the metabolic effects of training that have been discussed previously are based on the premise that the work performed during the 1st and 7th day of training was (at least) the same. It is possible, since the training resulted in an increase in peak cadence in the Wingate test, that cadence during the 10 s sprints was greater on the 7th compared to the 1st day of training. Cadence is a variable that contributes to the amount of work performed. If the amount of work performed on the 7th day was greater than that on the 1st day, then the metabolic changes that were observed are even more remarkable.

Although, at this point, mechanisms for reduced accumulation of AN degradation products in the plasma after sprint training need further investigation, it is noteworthy that in this study, the training had a pronounced effect on metabolism in only 7 days. Most training studies only measure adaptations after 5 to 6 weeks.

4.3.4 Exercise performance

Improvements in high-intensity short duration performance with high-intensity short duration training have been previously documented (Houston and Thomson, 1977; Nielsen et al., 1994; Nevill et al., 1989; Stathis et al., 1994). The 10 to 12% increase in peak power in the Wingate test is comparable to the 12% improvement reported by Nevill et al. (1989) in a 30 s treadmill sprint test but less than the 25% increase in a 30 s Wingate after 7 weeks of sprint training (Linossier et al., 1993). The mechanisms responsible for this improved performance are not clear. The most obvious, is that motor coordination improved (by familiarity) with training such that the subjects are able to increase cadence. Peak power is a function of both peak cadence and the workload applied (which in this test was constant), thus if peak cadence increases peak power will increase accordingly. Alternatively, the improved performance in the 30 s sprint may be related to the decreased plasma accumulation of ammonia and other purines after training. The latter is associated with less or delayed perturbation of cellular ATP homeostasis, and less accumulation of ADP, P_i, and H^+ which are known to be potential fatiguing agents (Cooke and Pate, 1990; Sahlin, 1992). Also, it has been suggested that exercise-induced hyperammonemia may be involved in the mechanism of muscle fatigue by its effect on the central nervous system (e.g motor incoordination) (Banister and Cameron, 1990); and thus the comparatively lower plasma NH_3 after training may have contributed to a delayed onset of muscular fatigue.

In addition, performance was improved despite subjects having a reduced resting muscle concentrations of ATP and TAN. It is unknown whether performance would have been improved to a greater extent had ATP and TAN levels not been so reduced.

The lack of ergogenic effect of creatine supplementation on Wingate test performance may be related to two factors. Firstly, there was a low response of subjects to creatine ingestion, and secondly that the mainteanace dose was ineffective such that TCr in the

creatine group was not in fact higher than that in the placebo group by the end of training. In contrast, creatine loading with training had a significant ergogenic effect on peak sustained power output (PSPO). This may not be a highly reproducible finding considering that the improvement in PSPO in the creatine supplemented group was only 2.2% (+ 8 W) and the placebo groups' PSPO dropped by 1.4% (- 5 W). If this finding is not due to variability however, then it is particularly interesting considering that the average duration of a PSPO test is 8 to 10 mins - which is noticably longer (up to 8 mins) than any previously reported exercise that has been shown to improve with creatine supplementation (Balsom et al., 1993; Birch et al., 1994; Greenhaff et al., 1993; Lemon et al., 1995A;; Viru et al., 1993). This may be related to the observation that 57% of the creatine group made subjective evaluations of feeling "strong" or "good" during the training week in contrast to only one subject in the placebo group making a similar evaluation. This raises the interesting questions as to whether creatine: 1) has an acute ergogenic effect during high-intensity exercise soon after creatine ingestion; or 2) improves the capacity for sprint training and therefore the adaptation to it.

In contrast to the effect on the Wingate test parameters and PSPO, the week of high-intensity training did not increase the 1-hr distance performance significantly (although both groups increased the mean distance covered). This supports the theory of training specificity - that is, in order to improve high-intensity *endurance* performance, one must perform *sustained* high-intensity training. There is evidence that sustained high-intensity training improves endurance performance (Acevedo and Goldfarb, 1989). In the latter study, long distance runners improved their 10 km race time after 8 weeks of increased training intensity. The observation that glycolytic metabolism was comparatively unaffected by high-intensity training in the present study (compared to the pronounced effect on AN metabolism) is a possible explanation for the finding that 1-hr performance was unchanged. However, an increase of 1 km (as was shown in the placebo group),

although not statistically significant, may have become so with larger subject numbers, and would be of practical significance to performance in the field.

4.4 Conclusions

This study has shown that oral creatine supplementation results in an increase in muscle TCr content, the extent of which is strongly related to an individual's percentage of Type IIB fibres. A maintenance dose of 2 g of creatine per day is ineffective in maintaining TCr at a significantly higher level during a week of intermittent sprint training. Consequently, it is still unknown whether a significantly elevated muscle TCr content can attenuate the decrease in skeletal muscle ATP and TAN content after consecutive days of strenuous sprint training. Whether this decrease is a result of the stress of the training, or an adaptation to the training cannot be concluded from this study. The training did, however, result in an attenuation in the accumulation of plasma products of adenine nucleotide degradation (NH_3, hypoxanthine and urate) during and after exercise. Also, performance in a 30 s Wingate cycle test improved significantly after training, although the training did not improve 1-hr cycle distance performance. The ergogenic effect of creatine supplementation during sprint training on performance indices cannot be effectively evaluated from this study since TCr content did not remain significantly elevated during the week of training. In addition, the relatively low subject numbers and marginal changes in endurance performance preclude firm conclusions.

CHAPTER 5:

THESIS CONCLUSIONS

In recent years the practise of oral creatine supplementation has received considerable interest from both scientists and athletes. Although earlier studies showed that creatine supplementation resulted in increased muscle TCr levels and had an ergogenic effect on short duration high-intensity exercise, more recent reports showing varied responses in the change in TCr after supplementation and no improvements in exercise performance have made the topic controversial. This study complements current literature confirming that oral creatine supplementation does in fact increase muscle TCr content, but emphasises that the extent of this increase is related to an individual's percentage of Type IIB fibres. This has important practical implications. An ergogenic effect of creatine supplementation should not be guarenteed to any athlete without prior examination of factors which may influence the efficacy of the athlete's "loading", in particular their muscle fibre composition.

For those individuals who respond positively to creatine supplementation, it is important to know the most physiologically effective, and cost effective ways to maintain high TCr stores over a longer duration of time. In this study the maintenance dose of 2 g of creatine per day that has been suggested previously was ineffective in maintaining TCr at a significantly higher level. This may have been due to (in part) the concurrent week of high-intensity sprint training, or perhaps another unidentified mechanism. This issue certainly warrants further investigation.

In support of previous work (Hellsten-Westing et al. 1993b; Stathis et al., 1994), it has been shown that consecutive days of intermittent sprint training results in a significant reduction in resting muscle TAN and ATP content. Although evidence for an alteration in AN metabolism during exercise was found, the exact mechanism for the reduction in resting stores cannot be concluded from this study. Possible theories are that the decrease is a result of the extreme metabolic stress of the sprint, or that it is an adaptation to the training. A more extensive examination of the breakdown compounds of AN metabolism and related enzyme activities is needed to determine the sites of adaptation and to provide support for such theories.

The question of whether the performance improvements after sprint training would have been even greater had muscle ATP or TAN concentration not been so reduced also remains to be answered. The hypothesis that an elevation in muscle TCr content through supplementation may attenuate the decrease in skeletal muscle ATP and TAN content after sprint training could not be resolved due to the ineffective maintenance of TCr levels during the training. This ineffective maintenance of TCr also prevented a reliable and valid evaluation of the ergogenic effect of increased TCr stores during intermittent sprint training. Both of these theoretical concepts should be resolved easily once an effective maintenance dose of creatine has been determined for these training conditions.

In addition to addressing novel theories, this study has provided support for established current literature in certain areas. These include the findings that sprint training 1) results in an attenuation in the accumulation of plasma products of adenine nucleotide degradation (NH_3, hypoxanthine and urate) during and after exercise; 2) improves sprint performance; and 3) does not improve high-intensity endurance performance.

In conclusion, this thesis has addressed a variety of current and controversial issues. Although some of the findings add to the established literature considerably, there are questions that have been left unanswered, and new theories have been raised which warrant further investigation.

It is recommended that future research include 1) the study of the efficacy of a maintenance dose of creatine without concurrent sprint training, and 2) the measurement of urinary creatinine excretion during this period to establish whether excretion is increased due to an elevated muscle TCr content. Once an effective maintenance dose of creatine has been established, a study similar to the present one ought to be done in order to validate whether an increase in TCr has an ergogenic effect on performance with sprint training. Finally, the mechanism for AN loss with intensive sprint training should certainly be examined by the measurement of AN, their breakdown products, and enzyme activities.

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APPENDICES

APPENDIX I: An example of the Subject Information Sheet.

This is some information regarding the laboratory trial you have volunteered to participate in. If you do not understand anything, please ask for further explanation. The muscle biopsy and blood sampling procedures will be explained to you in detail verbally. They will be performed by qualified medical personnel under sterile conditions.

The Tests:

- Peak sustained power output (PSPO) on a lab bike, where the workload is increased in stages until exhaustion..... lasts from 10 -15 min
- Wingate test on a lab bike, - an all-out maximal sprint for 30 seconds
- 1-hr trial on your own bike supported on a cyclosimulator in the laboratory, cover as much distance as possible in 1 hour (self-selected workloads)

Before each test:

- have a rest day or a light training session the previous day
- eat at least 4 hours before the test, and try to keep the meal and time of meal similar for all tests
- don't ingest any stimulants (eg caffeine), or alcohol
- take your last supplement tablet at least 8 hours before the test
- remember your pedals (and spanner) for the Wingate, PSPO, and training sessions; and your bicycle for the 1-hr trial
- check that your back tyre's inner tube has no previous punctures for the 1-hr test

The Familiarisation Week:

- to get you familiar with the tests and equipment

The Supplement Loading Week:

- you have either the creatine supplement or a placebo
- take 5 tablets at each of your 3 main mealtimes, and 5 tablets just before your training session (on your rest day, take the last 5 tablets just before going to sleep).....ie. you are taking 20 tablets / day
- dissolve the tablets in warm / hot water
- record time of ingestion on your log sheet
- any tablets not taken must be returned to the investigators

The Supplement Maintenance Week:

- take 2 tablets every day

The Sprint Training Week:

- training will be in the laboratory using the lab bike
- for 7 days, at the same time each day
- on day 1 and day 7 blood samples will be taken from a forearm vein
- 10 x 10 s sprints at a fixed workload, with 140 s recovery
- total time about 30 min

Training Record:

- record your training for 7 days after the first familiarisation 1-hr trial
- keep your training for the following week as similar to this as possible, and record again
- during the training week try to maintain your usual endurance riding, even if it is very low intensity

Dietary Record:

- maintain the same dietary intake for the 3 days prior to your second and third biopsies that you ingested for the 3 days prior to the 1st biopsy

APPENDIX II: An example of the Informed Consent.

I,_____, hereby consent to participate in a research project at the MRC/UCT Bioenergetics of Exercise Research Unit, Department of Physiology, University of Cape Town Medical School.

I understand that the project will involve multiple laboratory visits over a 3-week period. On these occasions I will be required to undergo a variety of exercise tests including 3 peak sustained power output tests, 3 one-hour cycling trials, and three 30 s Wingate tests. I understand that I will be required to complete one week of high-intensity training involving multiple 10 s sprints each day. In addition, I understand that on two occasions blood samples will be taken during and after the exercsie tests.

Furthermore, I understand that I will be required to have muscle biopsies on 3 separate occasions. I realise that this procedure is invasive and requires a sample of muscle to be taken from my leg by a qualified medical doctor using local anaesthetic and normal sterile procedures.

I fully understand the risks associated with this project which have been outlined in the information sheet and explained to me. I understand that I am free to withdraw from the study at any time should I so choose.

Signed_____ Date_____

Investigator_____

Supervisor_____

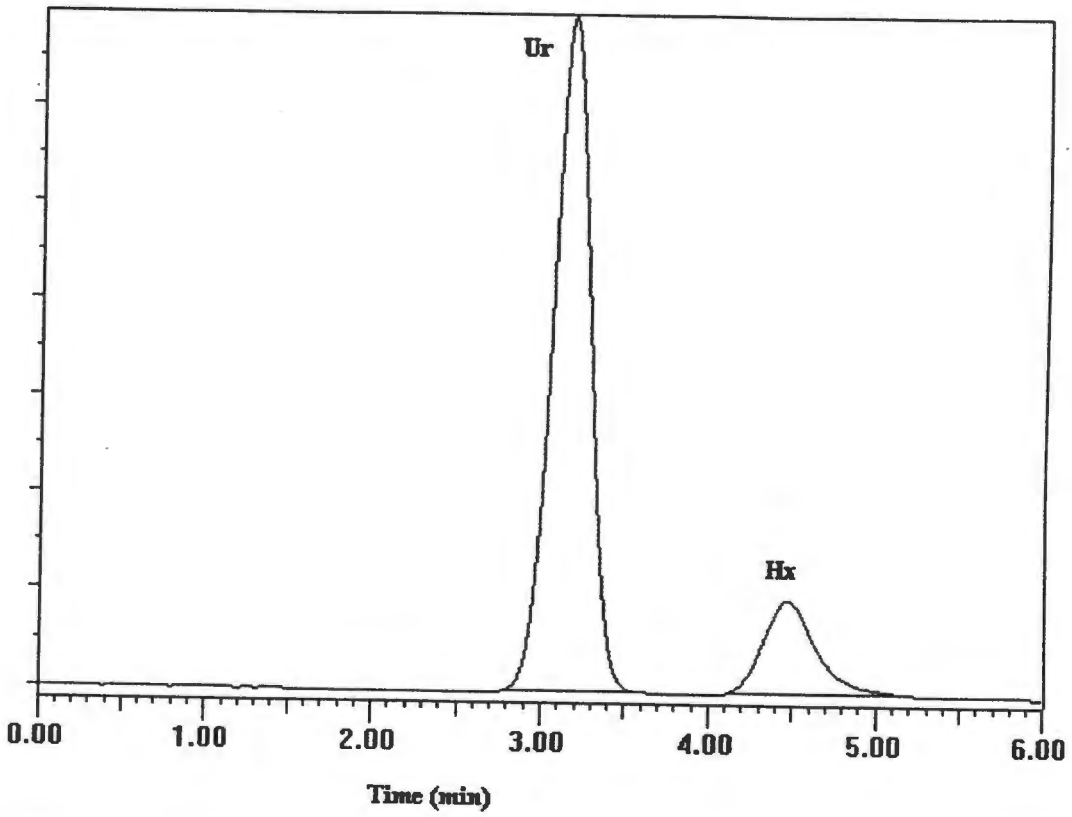
APPENDIX III: Rating of Perceived Exertion (Modified Borg Scale).

Rating of Perceived Exertion

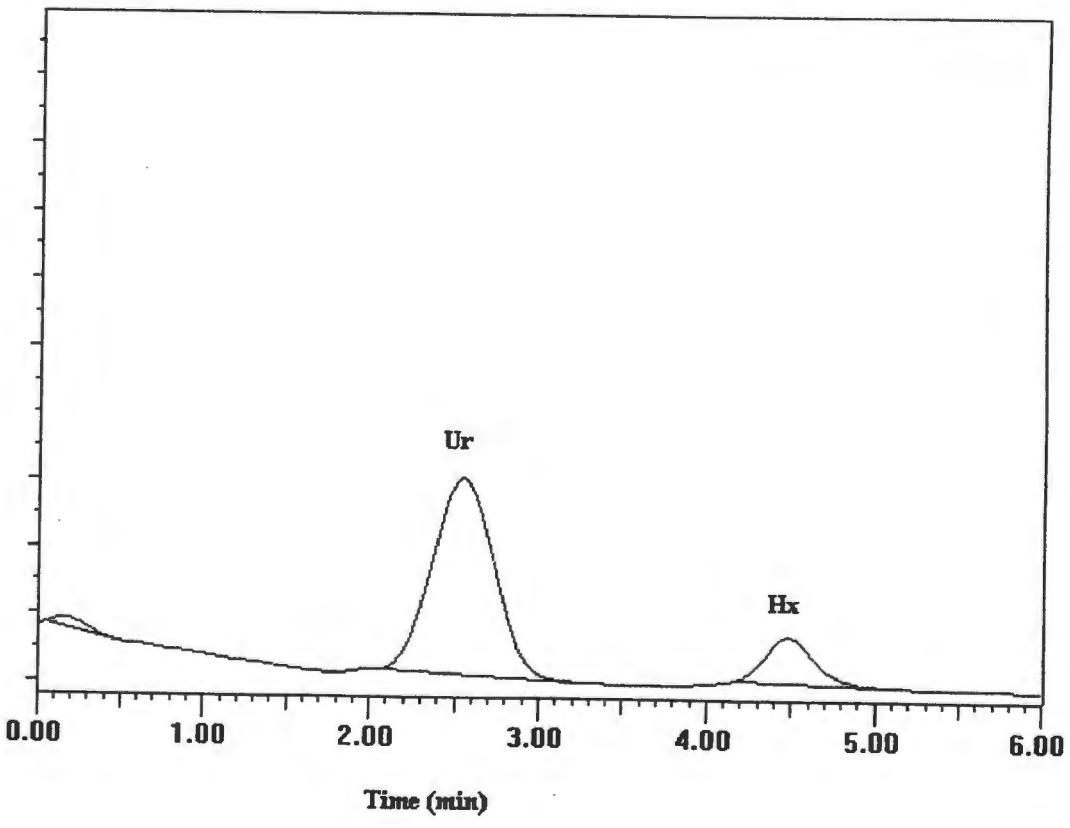
6	
7	fairly light
8	
9	somewhat hard
10	
11	hard
12	
13	very hard
14	
15	very, very hard
16	
17	extremely hard
18	
19	exhausting
20	

APPENDIX IV: A chromatogram of both a standard solution (A) and an extracted plasma sample solution (B) from the analysis of plasma urate and hypoxanthine using HPLC (λ = 249nm).

A.

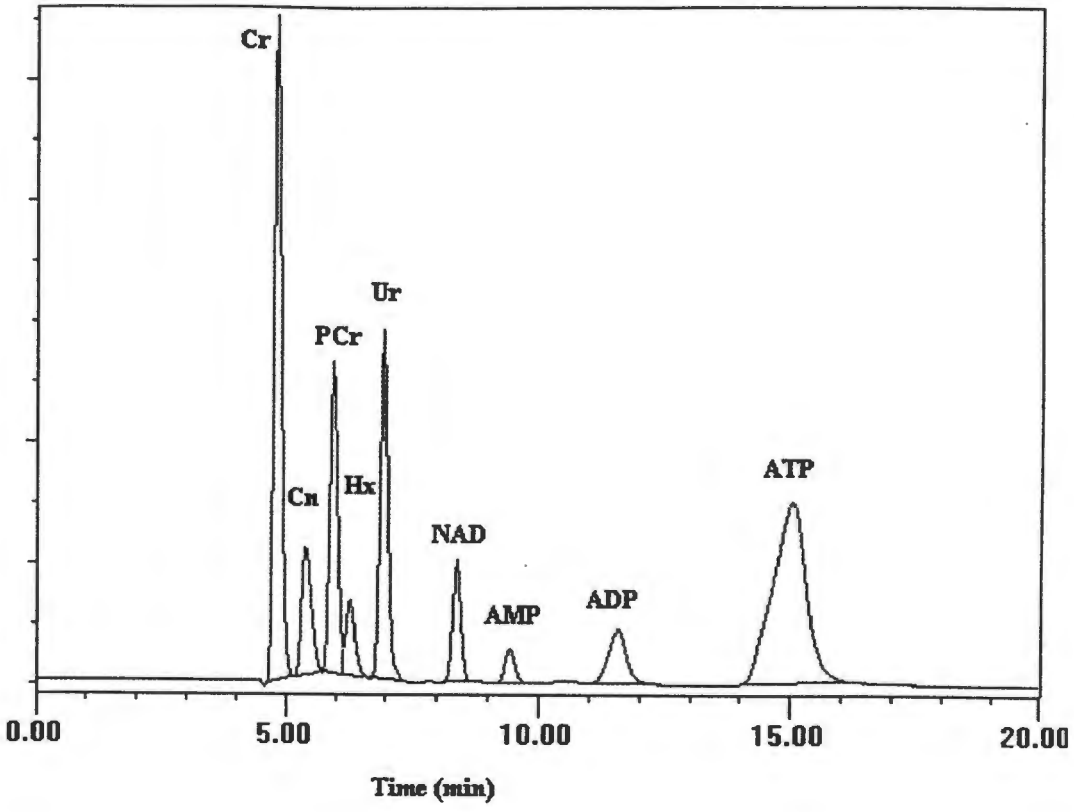


B.

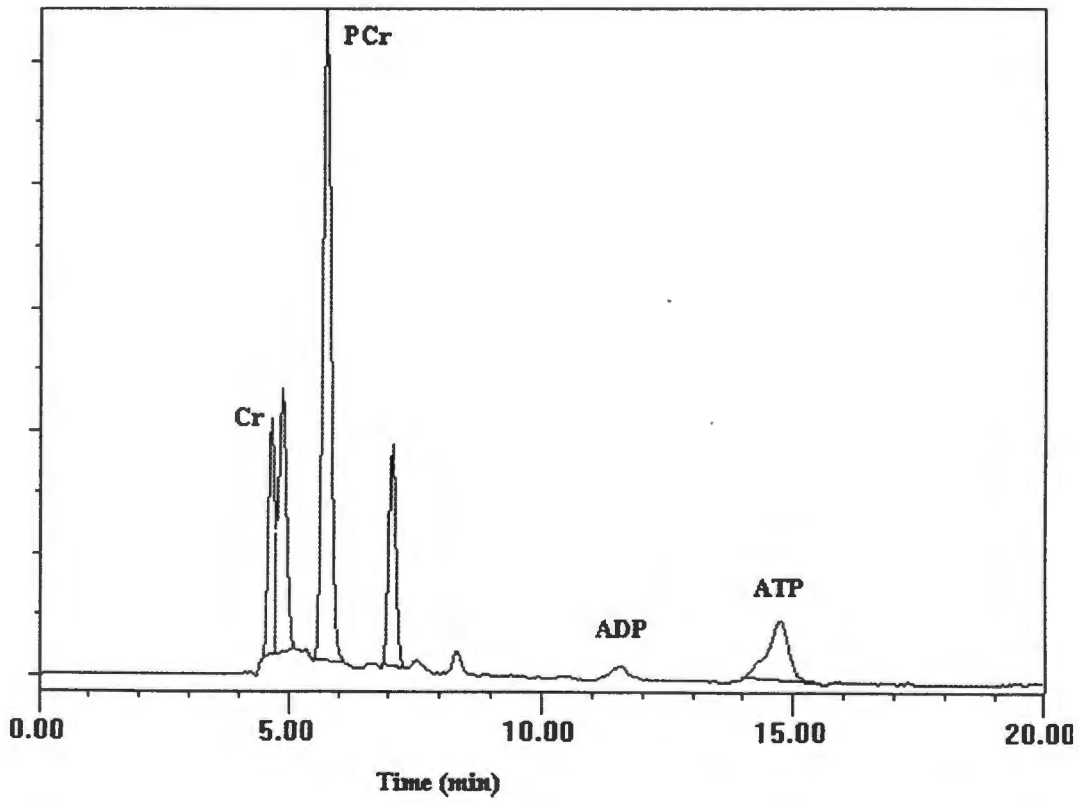


APPENDIX V: A chromatogram of both a standard solution (A) and an extracted muscle sample solution (B) from the analysis of adenine nucleotides and creatine compounds using HPLC (λ = 210nm and 260nm).

A.



B.



APPENDIX VI: Plasma ammonia accumulation (μmol/l) on the 1st and 7th day of sprint training for both the placebo and creatine groups.

	Placebo group		Creatine group	
	1st day	7th day	1st day	7th day
Rest	43.2 ± 5	54.6 ± 13.7	41.2 ± 4	48.8 ± 5
Sprint bout 5	143.8 ± 26	139.0 ± 33	141.5 ± 18	111.2 ± 12
Sprint bout 10	198.0 ± 25	186.0 ± 24	194.5 ± 16	135.7 ± 22
5 min recovery	181.8 ± 29	133.4 ± 10	164.7 ± 12	120.2 ± 17
10 min recovery	144.5 ± 22	90.4 ± 14 *	134.0 ± 12	100.7 ± 17 *
20 min recovery	97.6 ± 19	65.0 ± 4	93.8 ± 11	76.0 ± 12
30 min recovery	76.2 ± 6	57.4 ± 5	79.3 ± 9	67.5 ± 12

Values are means ± SEM. * Significantly different from 1st day of sprint training. No difference between the groups.

APPENDIX VII: Plasma hypoxanthine accumulation (μmol/l) on the 1st and 7th day of sprint training for both the placebo and creatine groups.

	Placebo group		Creatine group	
	1st day	7th day	1st day	7th day
Rest	1.7 ± 0.3	2.4 ± 1.4	1.0 ± 0.2	1.4 ± 0.4
Sprint bout 5	23.3 ± 4.4	7.8 ± 4.2 *	19.2 ± 2.7	4.2 ± 1.3 *
Sprint bout 10	57.9 ± 8.9	23.7 ± 8.2 *	48.5 ± 6.9	19.4 ± 4.8 *
5 min recovery	60.2 ± 9.0	25.9 ± 7.6 *	49.4 ± 4.1	22.1 ± 5.7 *
10 min recovery	56.5 ± 9.9	28.6 ± 8.2 *	52.8 ± 4.2	23.3 ± 6.2 *
20 min recovery	50.8 ± 10.1	21.2 ± 7.5 *	52.0 ± 2.4	21.2 ± 7.2 *
30 min recovery	41.4 ± 9.2	14.9 ± 5.3 *	44.4 ± 2.8	17.7 ± 7.1 *

Values are means ± SEM. * Significantly different from 1st day of sprint training. No difference between the groups.

APPENDIX VIII: Plasma urate accumulation (μmol/l) on the 1st and 7th day of sprint training for both the placebo and creatine groups.

	Placebo group		Creatine group	
	1st day	7th day	1st day	7th day
Rest	283.7 ± 23	306.1 ± 18	327.0 ± 20	329.1 ± 31
Sprint bout 5	299.1 ± 20	305.5 ± 18	330.0 ± 18	332.1 ± 32
Sprint bout 10	331.9 ± 22	312.4 ± 16	311.9 ± 49	362.2 ± 50
5 min recovery	328.0 ± 22	307.7 ± 17	359.8 ± 23	331.6 ± 24
10 min recovery	387.9 ± 40	330.0 ± 19 *	386.8 ± 14	327.9 ± 13 *
20 min recovery	424.2 ± 35	355.5 ± 20 *	427.8 ± 20	359.5 ± 19 *
30 min recovery	438.0 ± 40	370.1 ± 23 *	459.2 ± 16	368.6 ± 13 *

Values are means ± SEM. * Significantly different from 1st day of sprint training. No difference between the groups.

APPENDIX IX: Plasma lactate accumulation (mmol/l) on the 1st and 7th day of sprint training for both the placebo and creatine groups.

	Placebo group		Creatine group	
	1st day	7th day	1st day	7th day
Rest	1.8 ± 0.1	1.8 ± 0.3	1.9 ± 0.2	1.9 ± 0.1
Sprint bout 5	12.7 ± 1.4	13.2 ± 0.6	15.2 ± 0.6	14.8 ± 0.6
Sprint bout 10	16.2 ± 1.3	17.0 ± 1.2	17.8 ± 0.7	18.3 ± 1.1
5 min recovery	15.0 ± 1.8	16.5 ± 1.4	18.1 ± 0.5	18.2 ± 1.3
10 min recovery	14.2 ± 2.1	14.3 ± 1.4	16.0 ± 0.9	17.0 ± 1.4
20 min recovery	10.6 ± 1.9	10.2 ± 1.5	13.3 ± 1.0	13.6 ± 1.8
30 min recovery	7.3 ± 1.5	7.7 ± 1.3	10.8 ± 0.9	10.4 ± 1.7

Values are means ± SEM. No difference between 1st day and 7th day of sprint training, or between the groups.

APPENDIX X: Peak heart-rate (bpm) after each 10 s sprint bout on the 1st and 7th day of sprint training for both the placebo and creatine groups.

	Placebo group		Creatine group	
	1st day	7th day	1st day	7th day
Bout 1	163.8 ± 7	160.2 ± 9	163.3 ± 6	165.8 ± 4
Bout 2	168.0 ± 7	159.4 ± 6	164.3 ± 6	163.8 ± 6
Bout 3	169.0 ± 6	162.6 ± 3	164.7 ± 6	165.3 ± 5
Bout 4	168.3 ± 5	164.0 ± 3	167.8 ± 5	164.8 ± 6
Bout 5	169.4 ± 4	168.0 ± 3	166.8 ± 6	165.7 ± 5
Bout 6	164.8 ± 3	163.8 ± 3	163.3 ± 3	166.5 ± 5
Bout 7	167.0 ± 4	165.8 ± 4	162.3 ± 4	165.0 ± 5
Bout 8	165.2 ± 3	164.8 ± 3	163.2 ± 4	165.2 ± 5
Bout 9	164.6 ± 3	166.2 ± 3	163.2 ± 4	163.8 ± 5
Bout 10	167.2 ± 3	164.6 ± 4	162.8 ± 4	164.3 ± 4

Values are means ± SEM. No difference between 1st day and 7th day of sprint training, or between the groups.

APPENDIX XI: Theoretical calculation of the amount of the total creatine pool that is excreted as creatinine in both a normal subject and a creatine-loaded subject.

According to Crim et al. (1976), 1.6% of the total creatine pool is lost as creatinine per day. Based on the fact that 95% of the total creatine pool in in the skeletal muscle, this calculation makes the assumption that the total creatine pool is equivalent to the skeletal muscle creatine pool.

a) In normal unloaded muscle (placebo group)

TCr = 120 mmol/kg dm
= 24 mmol/kg wwt
= 960 mmol (If 58% of a 70 kg trained male is muscle mass, then muscle weight = 40 kg)

Amount excreted = 0.016 x 960
= 15.4 mmol per day
= **2.3 g per day** (molecular mass of creatine = 150)

b) In creatine loaded muscle (creatine group)

TCr = 150 mmol/kg dm
= 30 mmol/kg wwt
= 1200 mmol (If 58% of a 70 kg trained male is muscle mass, then muscle weight = 40 kg)

Amount excreted = 0.016 x 1200
= 19.2 mmols per day
= **2.9 g per day** (molecular mass of creatine = 150)