

Factors Influencing Short Duration, **High-Intensity Endurance Cycling**

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Declaration

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

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Abstract

A 5 km cycling time trial (TT) demands high, sustained muscle power output and substantial oxidative and glycolytic energy delivery. The aims of this study were to firstly use the novel approach of using Peak Sustained Power Output (PSPO) as a predictor of cycling performance for variable fixed-workload testing and 5 km time trialing and whether oral creatine supplementation would affect 5 km time trial performance and metabolism. The effect of oral creatine supplementation with 20 g/day for 7 days on 5 km time trial performance and metabolism after a random-variable workload protocol (stochastic test) was investigated in a single-blind placebo controlled study. Thirteen trained male cyclists volunteered for the study that was approved by the University of Stellenbosch Research Ethics Committee. We hypothesized that Cr supplementation would affect time trial performance as well as the appearance of breakdown products of adenine nucleotides in the plasma. **Baseline:** the subjects' peak power output was measured and they underwent a baseline stochastic test followed immediately by a 5 km time trial (ST1) and on a different day, a single 5 km time trial test (T1) was undertaken. **Study 1:** In the following week the subjects repeated the stochastic test with 5 km time trial (ST2) and on a different day a 5 km time trial (T2). During T2 blood samples were taken at regular intervals as well as during recovery. A muscle biopsy was taken after T2 in the recovered state. The major performance predictors were the 5-km time trials (TT1 and TT2) with a coefficient of variation between the thirteen trained male cyclists of 0.6%. The 5 km time trials in the fatigued state (ST1 and ST2) had a coefficient of variation of 0.7%. **Results:** There was a significant difference between 5 km TT performed fresh and 5km TT performed fatigued ($P=0.0001$). The decrement in time ranged between 1.0 sec to 38.0 sec. The relationship between two different high intensity endurance performance tests: PSPO and 5 km TT (TT mean) had a correlation of $r=-0.79$ $P<0.01$. The correlation between PSPO and the 5 km TT performed in the fatigued condition (ST mean) was $r= -0.60$ $P<0.05$. There was also a relationship between age and PSPO ($r= 0.73$; $P<0.05$). Age showed a good negative relationship with TT mean ($r=-0.71$; $P<0.05$). Mass and PSPO were also correlated ($r=0.85$; $P<0.01$). Plasma lactate concentrations were significantly different from rest at all other time points $P<0.0001$ up to 20 minutes post-exercise. Hypoxanthine ($P<0.0001$) and urate ($P=0.05$) concentrations

were also significantly different from rest at all other time points. There was a significant change in plasma hypoxanthine concentrations over time ($P < 0.0001$). There was a significant correlation between plasma lactate concentration at time 0 in recovery and % Myosin Heavy Chain (MHC) I ($r = 0.59$, $P < 0.05$) and % MHC IIa ($r = -0.61$, $P < 0.05$). Area under the curve for hypoxanthine showed significant relationships of $r = -0.53$ ($P = 0.05$) and $r = 0.56$ ($P < 0.05$) respectively for %MHC I and %MHC IIa. Study 2 - Supplementation: following T2 the subjects received Cr or placebo powder containing sachets to be ingested 4 times daily for the next week with carbohydrate also provided. Post-supplementation testing: the subjects returned and conducted another variable fixed-workload test with 5 km time trial (ST3) as well as a single 5 km time trial (T3). They once again underwent the same blood sampling routine and had another muscle biopsy in the rested state. The average of ST1 and ST2 was used as the major measure for performance under fatigued conditions and TTmean. Results: there was a significant difference between 5 km TT performed fresh and 5 km TT performed fatigued ($P = 0.0001$). Plasma lactate, hypoxanthine and urate samples were taken at the post-loading 5 km time trial (TT3). The levels of these plasma metabolites were compared to the concentrations of those sampled at TT2 and between the creatine and placebo groups. The plasma hypoxanthine levels were significantly different from rest for both the creatine and placebo groups ($P < 0.0001$). Plasma urate had significant change in concentrations over the time points ($P < 0.005$). Another significant difference was found between the creatine and placebo groups for the pre- and post tests conducted ($P < 0.005$). The creatine group showed a non-significant increase (7%) in mean total intramuscular creatine concentration. No significant differences were found in the mean values for total nucleotide concentration pre- and post loading in the creatine and placebo groups. Conclusions: The 5 km performance test resulted in high values for plasma lactate, hypoxanthine and urate, an indication of fatigue induced by this performance test. Oral creatine supplementation did not improve performance significantly in the variable fixed-workload protocol 5 km time trials or the individual 5-km time trials and had an effect on adenine nucleotide metabolism in both the variable fixed-workload protocol 5-km time trial and individual 5 km time trial performance tests.

OPSOMMING

Die 5 km fietsry tyd toets (TT) verg hoëvolgehoue, spierkrag en wesenlike oksidatiewe en glikolitiese energie verskaffing. Die doelwitte van hierdie tesis was eerstens om 'n nuwe fietsergometer toets te ontwerp vir die voorspelling van kompetisie prestasie. Die toets was 'n veranderlike, voorafvasgestelde werkladingstoets (VVWT). Dit is vergelyk met 'n maksimale volgehoue kraguitsettingstoets (MVKT) en 'n 5 km TT. Daarna is bepaal of kreatien supplementasie 'n effek sou hê op enige van hierdie oefeningstoetse of metabolisme tydens die 5 km TT. Die supplementasie eksperiment was 'n enkelblinde, plasebo-gekontroleerde studie ontwerp. Dertien manlike fietsryers het vrywilliglik deelgeneem. Resultate: Daar was 'n statisties betekenisvolle verskil tussen die 5 km TT wat vars onderneem is en die wat direk na die VVWT onderneem is ($P=0.0001$). Daar was ook 'n betekenisvolle korrelasie tussen die twee verskillende hoë intensiteit korttydsvak oefeningstoetse ($r=-0.79$ $P<0.01$ vir TT en MVKT). Plasma laktaat konsentrasies was betekenisvol verhoog met vergelyking van die monsters geneem tydens rus en all ander onteledings tydspunte ($P<0.0001$) tot en met 20 minute na oefening. Hypoxantien ($P<0.0001$) en uraat ($P=0.05$) konsentrasies was ook betekenisvol verskillend van rus by alle ander tydspunte. Daar was 'n betekenisvolle korrelasie tussen plasma laktaat direk na oefening en die % Myosien Swaarketting (MHC) I ($r=0.59$, $P<0.05$) en % MHC IIa ($r=-0.61$, $P<0.05$). Studie 2 – Na supplementasie was daar geen verskil in oefeningsprestasie nie maar wel in plasma metaboliet waardes van nie. Gevolgtrekkings: Die 5 km TT het baie hoë waardes vir plasma laktaat tot gevolg gehad, asook hypoxantien en uraat, 'n indikasie van die hoë mate van vermoeienis deur hierdie oefeningtoets berwerkstellig. Kreatine supplementasie kon nie oefeningsprestasie verbeter nie.

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Abbreviations

PSPO – Peak Sustained Power Output
MHC – Myosin Heavy Chain
ATP – Adenosine 5' - triphosphate
ADP – Adenosine 5' - diphosphate
AMP – Adenosine 5' - monophosphate
TCr – Total creatine
PCr – Phosphocreatine
Cr – Creatine
CP – Creatine Phosphate
VO₂max – Maximal Oxygen Uptake
Na⁺ – Sodium ions
K⁺ - Pottasium ions
ST – Slow twitch
FT – Fast Twitch
MATPase – Myosin Adenosine Triphosphatase
Pi – inorganic phosphate
Sec – seconds
G6-P – Glucose-6-phosphate
G1-P – Glucose-1-phosphate
F6-P – Fructose-6-phosphate
PFK – Phosphofructokinase
PEP – Phosphoenolpyruvate
PNC – Purine Nucleotide Cycle
IMP – Inosine 5' - monophosphate
Acetyl-CoA – Acetyl Coenzyme A
GDP – Guanosine diphosphate
GTP – Guanosine triphosphate
FAD – Flavin Adenine Dinucleotide
AnGP – Anaerobic glycolytic pathway

FFA – Free fatty acids
SNS – Sympathetic nervous system
Min – minutes
RER – Respiratory Exchange Ratio
GI – Glycemic index
BCAA – Branched Chain Amino Acids
LT – Lactate Threshold
NMR – Nuclear Magnetic Resonance
SAM – S-adenosylmethione
GAA – Guanidinoacetic acid
Crea T – Creatine transporter
CK – Creatine Kinase
GPA – 3-guanidinopropionate
CHO – Carbohydrate
HPLC – High-Performance Liquid Chromatography
SD – Standard Deviation

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CHAPTER 1: Introduction

Many sports require the athlete to repeatedly engage in high-intensity exercise with varying amounts of recovery time between bouts. Most of the energy for short bouts of high-intensity exercise is derived from anaerobic sources and therefore the ability to recover during rest periods is essential for success.

Physical activity results in the splitting of ATP in the exercising muscles. Because the ATP stores are very limited the ATP broken down must be resynthesized continuously at the same rate as it is used. At moderate exercise intensities, this resynthesis is accomplished by aerobic processes. High-intensity exercise where the ATP-turnover rate exceeds the maximal power of the oxygen transporting system is in addition heavily dependent on anaerobic ATP-forming processes. The ability to exercise at high intensities is therefore dependent on the capacities of both the aerobic and anaerobic processes (Medbo and Tabata, 1989).

Exercise of high intensity may cause large increases in the adenosine 5'-triphosphate (ATP) concentrations of skeletal muscle (Cheatham *et al.*, 1986). Simultaneously an increased production of adenosine 5'-diphosphate (ADP) activates adenylate kinase which together with adenosine 5'-monophosphate deaminase maintains the balance of the adenine nucleotide pool. The inosine monophosphate produced subsequently undergoes oxidation and hypoxanthine is formed and has been shown that increased concentrations of hypoxanthine in plasma following maximal short term running (Hellsten-Westling *et al.*, 1989). Hypoxanthine is then oxidized to urate, an irreversible reaction in human tissue.

There has been a great deal of excitement amongst the scientific research family and athletes about the dietary supplement creatine. Scientific research has shown that creatine is not just an energy source that powers muscles, but it is more. Although creatine has been known to man since 1832, its presence in athletes as a performance enhancer is relatively new. Creatine has become the athlete's most important nutritional ergogenic aid because (Juhn, 1999):

- There is a higher total creatine concentration [TCr] in skeletal muscle, including both phosphocreatine [PCr] and free creatine [Cr], allowing for enhanced anaerobic ATP production during maximal exercise.
- It enhances and accelerates the resynthesis of PCr during recovery periods between bouts of intense exercise.
- Promotes significant increases in muscle size (lean body mass).

It should however be noted that the creatine phosphate (CP) reaction is anaerobic (Bessman and Geiger, 1981), meaning that oral creatine supplementation is potentially ergogenic only for activity that has a high anaerobic component, but not for endurance activity (Balsom *et al.*, 1993a). The possibility exists, however, that creatine may be ergogenic for endurance events that involve intermittent bursts of anaerobic activity.

The purpose of this investigation was to study the performance of endurance cyclists in high intensity, short duration endurance cycling. Another aim of the study was to investigate the metabolic changes in the subjects performing such physical activities as well as the effect of oral creatine supplementation on such a cycling protocol.

Chapter 2: Predictors of Cycling Performance

“ There seems to be no formula that determines a champion. Champions are born and then made. This applies to all of us. We all need to work to be our best.”

–Connie Carpenter-Phinney –

Competitive road cycling is very demanding physiologically and psychologically. A cyclist is called upon to have stamina, strength and bursts of speed. The first challenge to creating a successful training program and becoming a successful cyclist is to have a better understanding of the required physiological and biochemical factors that affect performance. Thus the factors that affect performance in cyclists and that will be monitored in the research studies in this thesis, are the cardio-respiratory system, body mass, muscle composition and muscle metabolism.

2.1 The Cardio-Respiratory System

The key factor to performance in the cardio-respiratory system is maximal oxygen utilization or $VO_2\text{max}$. It is traditionally defined as the maximal rate at which oxygen can be taken up and used by the body during heavy, exhaustive aerobic exercise. It is generally accepted as a good measure of the peak function of the cardiovascular system of the test subject for the particular test protocol and often interpreted as an index of cardio-respiratory fitness (Katch *et al.*, 1982). Because oxygen is needed to convert fuel (carbohydrates and fats) into energy, the more oxygen you can use, the more energy you can provide and the more energy you can produce the less likely you are to fatigue during cycling.

There are other determinants of maximal oxygen uptake. In the broadest sense, it is determined by the maximal cardiac output and maximal arterio-venous oxygen partial pressure difference, $\max \text{PaO}_2 - \text{PvO}_2$ difference, as expressed in the following equation (Snell and Mitchell, 1984):

$$\text{VO}_2\text{max} = \text{max cardiac output} \times \text{max PaO}_2 - \text{PvO}_2 \text{ difference}$$

One of the most fundamental beliefs in Exercise Physiology is that performance in endurance exercise has a cardiovascular limitation, but it is possible that there is not a cardiovascular limitation to exercise performance and that VO_2max is not a measure of a physiological upper limit, rather it is a measure of the peak VO_2 at termination of a particular test due to exhaustion. Thus, an alternative mechanism is needed to explain exhaustion during maximal exercise performance. Noakes, (1988), proposed that the factors limiting exercise might be better explained in terms of failure of muscle contractility, which may be independent of tissue oxygen deficiency. An increase in oxygen consumption is due to increased workload and increased muscle cross-bridge activity. This theory implies that the peak power output of the legs generated in the final workload of the test is the more important variable instead of VO_2max .

Because there is a relationship between peak sustained power output (PSPO) and VO_2max , shown by Noakes (1988), a theoretical VO_2max can be calculated using a specific formula incorporating Peak Sustained Power Output (PSPO). The formula is as follows:

$$\text{VO}_2\text{max (calc)} = 0.01141 \times \text{PSPO (W)} + 0.435$$

(then, multiply by 1000 to convert the answer to ml/min/kg)

Elite road cyclists have some of the highest recorded values for maximal oxygen uptake. Elite male and female cyclists range between 75 and 90 ml/kg/min and between 60 and 70 ml/kg/min respectively.

Several studies have indicated the importance of maximal muscle power as a predictor of athletic potential (Noakes *et al.*, 1990; Hawley and Williams, 1991). If PSPO during progressive exercise to exhaustion, or during maximal exercise of short duration is a better predictor of endurance performance than VO_2max , the need to measure VO_2max may be questioned. In a study by Hawley and Noakes, (1992), there were two important findings. Firstly, there was a highly significant relationship between VO_2max and the PSPO a cyclist could sustain during the final workload of the maximal test ($r = 0.97$, $P < 0.0001$). The second important finding of this study was the highly significant relationship between PSPO and the performance time in a 20 km time trial ($r = -0.91$, $P < 0.001$), so that PSPO could explain 82% of the variance in times for a 20-km time trial.

2.2 Body Mass

Body mass is also an important factor in road cycling. It is important to relate body mass to PSPO. In cycling, carrying extra mass is a definite disadvantage, especially when climbing a hill and the power to weight ratio plays an important role. However a cyclist also needs a specific amount of muscle mass for the power aspects of the sport, such as time trials and sprints. Body mass alone is not an appropriate indicator of performance potential. Body composition (the amount of fat and muscle) is also important.

Peak sustained power output can be considered as a dynamic measure of muscle mass contributing to cycling performance. It is often related to body mass, i.e. PSPO/kg. However, there is a poorer prediction for time trial performance when PSPO is related to body mass. When PSPO was expressed relative to a cyclist's body mass, the correlation between PSPO and the 20 km time decreased to 0.68 ($P < 0.01$) (Hawley and Noakes, 1992). This can probably be attributed to the flat time trial course used. Previous studies have shown that body size and mass have an effect on energy cost of cycling. With a flat course, wind resistance is by far the most important factor in slowing the cyclist down and increasing the energy cost. Larger cyclists have more body surface and thus more wind resistance than their smaller counterparts and thus need more energy to overcome the higher wind resistance. But larger cyclists have greater absolute capacity to convert

energy into power. So which factor wins? The disadvantage of a little extra wind resistance is more than outweighed by the extra energy supplied by large cyclists on flat surfaces (Swain *et al.*, 1987). The advantage is lost when cycling uphill where gravity plays the biggest role.

Peak sustained power output tests were conducted in this research study and were used to predict time trial performance over a short course (5 km) and to determine sub-maximal workloads for the variable fixed-workload cycling protocol. This points to another advantage of PSPO over $VO_2\text{max}$. In contrast to $VO_2\text{max}$, which may drift during exercise at a fixed workload, peak power output remains fixed when using an electromagnetically braked cycle ergometer. Therefore, the workloads do not need to be altered during a test to maintain a fixed $VO_2\text{max}$ and baseline tests can be exactly repeated later.

2.3 Muscle.

In addition to having a high $VO_2\text{max}$, the endurance and speed of the cyclist depends largely on the muscles' ability to produce energy and force. Force originates at the molecular level from the interaction of the major muscle proteins, actin and myosin in the presence of ATP and Ca^{2+} (reviewed by Myburgh, 1994).

Intense contraction, particularly with large muscle groups, imposes a major strain on a wide variety of physiological systems. To generate the high force levels accompanying intense activity, maximal or near maximal activation of all the synergistic muscles is a fundamental requirement (Green, 1997). In the case of cycling, seven different synergistic muscles are recruited, their activation pattern being highly ordered and dependent on the limb and pedal position during cycling (Green and Patla, 1992). At the level of the individual muscle, maximal tetanic force for maximal work depends on full recruitment of all motor units at high firing frequencies (DeLuca, 1985). In terms of the individual muscle cell, a successful response to the high firing frequencies is critically

dependent on the sarcolemma and T-tubule system being able to regenerate action potentials at high frequency to the interior of the cell. The ability to sustain multiple action potentials is primarily dependent on the ability to get the potassium ions (K^+) back into the cell from the interstitial space and to expel the excess sodium ions (Na^+), which enter during the action potential, back to the interstitial space. Control of the electrochemical gradients is primarily under the control of an electrogenic pump, which expends energy in the form of ATP to pump both Na^+ and K^+ against their concentration gradients. The enzyme involved, for hydrolyzing the ATP and producing the necessary energy for this process is embedded in the membrane and is called the Na^+/K^+ - ATPase (Clausen and Nielsen, 1994).

The movement of these molecules and subsequent depolarization of the sarcolemma and t-tubules is believed to unblock the calcium release channel in the sarcoplasmic reticulum, allowing calcium (Ca^{2+}) to escape to the surrounding cytoplasm and result in an increase in the concentration of free Ca^{2+} . The generation of high force levels depends on the Ca^{2+} signal begin translated via the regulatory proteins, troponin and tropomyosin, leading to formation of the actomyosin bond and subsequent conformational change from a weak binding to a dominant strong binding, force-generating state (Moss *et al.*, 1995). Weak to strong binding is mediated via activation of an ATPase, located in the myosin heavy chains (MHC), which allows for the generation of free energy via ATP hydrolysis and release of metabolic by-products, ADP and inorganic phosphate. As with sarcolemma and T-tubules, continued excitation-contraction coupling relies on a cyclical elevation of free Ca^{2+} for maximal activation of the myofibrillar apparatus.

2.3.1 Muscle fibre types.

The functional, metabolic and molecular characteristics of skeletal muscle are quite diverse. This diversity is in part due to the existence of different muscle fibre types having particular characteristics and found in different proportions within a particular muscle. Three major fibre types can be identified on the basis of the intracellular content of myosin heavy chain isoforms (Staron and Johnson, 1993). These fibres can be

distinguished with the use of staining techniques revealing the sensitivity of the myofibrillar myosin ATPase enzyme exposed to various pH conditions (Brooke and Kaiser, 1970). Type I are also called slow twitch (ST) fibres. Their metabolism is also specifically adapted to prevent fatigue. For example Type I fibres have a high content of myoglobin (an oxygen-storing protein) and enzymes that favour aerobic energy production. The other major type of muscle fibre that has a lower aerobic capacity but a high anaerobic capacity is known as a Type II, or fast twitch (FT), fibre. Sub-categories of Type II fibers can also be found (e.g. IIA, IIB) (Simoneau, 1995). This fibre is normally found in high percentages in sprint and power athletes.

Is there a correlation between the percentage fibre type and myosin heavy chain content in human skeletal muscle? Using histochemical methods, Brooke and Kaiser, (1970), delineated three major fibre types (I, IIA and IIB) in adult human skeletal muscle based on the different pH sensitivities of their myosin adenosine triphosphatase (mATPase) activity. More recently, Perrie and Bumford, (1986), electrophoretically separated three myosin heavy chains (MHC I, MHC IIa and MHC IIb) in various human limb muscles, the expression of which in single fibres has been found to correlate with various mATPase-based fibre types (Staron *et al.*, 1991). However, this nomenclature should be altered to: I, IIA and IIX, since the so-called human IIB fibres actually express an MHC isoform equivalent to that of the rat IIX and not to that of the rat IIB (Smerdu *et al.*, 1994; Ennion *et al.*, 1995). Thus, after the discovery of a third fast isoform, the MHC IIX, it has gradually become evident that the human fast fibres named "IIB", according to evaluation by ATPase histochemistry, contained a transcript much more similar to that of rat IIX than of the rat IIB (Smerdu *et al.*, 1994; Ennion *et al.*, 1995). This lead to the suggestion that the human MHC isoforms should be termed I, IIa and IIX, to form consensus with the nomenclature for the rat MHC isoforms (Schiaffino and Reggiani, 1994). To distinguish between fibre type and MHC isoform, it has been suggested that fibre types should be called I, IIA and IIX, whilst MHC isoforms should be termed I, IIa and IIX (reviewed by Myburgh 1994).

In a study by Fry *et al.*, (1994), the authors determined the degree of association between the MHC content and the percentage area of major fibre types (types I, IIA and IIX) in the vastus lateralis muscle from men and woman, and assessed whether training has an effect on the relationship between these two variables. Their results showed that the vastus lateralis muscle in the human contains various mixtures of three MHC's. Endurance training caused a significant decrease in the percentage area of type IIX after 4 weeks and a significant increase in the percentage area of type IIA after 6 weeks for both men and woman. Similar changes were also found for the MHC content. MHC IIX significantly decreased after only 2 weeks and MHC Iia increased after 4-weeks. No changes in either percentage fiber type area or MHC content were found over time for the control men and women. Because various types of high intensity training cause fibre type transformations, a greater number of these fibres may occur as hybrid that are "caught in transition" and hence cause an under or over estimation of the amount of change when the change is determined by histochemistry rather than electrophoresis (Staron *et al*, 1991).

Thus, histochemical and electrophoretic techniques applied to human skeletal muscle establish a relationship between the percentage fibre type area and the MHC content. Because of the extensive use of mATPase histochemistry to distinguish skeletal muscle fibre types the use of this method makes comparison with other studies easier. However, since simple fibre typing (percentage calculation) does not take fibre area into account, electrophoresis may provide a more accurate assessment of muscle fibre type composition, especially in the absence of the equipment and software required for the determination of fibre areas. Moreover, muscle samples that contain large numbers of fibres coexpressing two MHC's in varying proportions (e.g. trained muscle) is definitely more accurately analyzed by gel electrophoresis.

2.3.2 Muscle fibre composition and cycling

Do cyclists need a specific muscle fibre type ratio? For a sprinter it would be ideal to have a high percentage of Type IIA or Type IIB. It is difficult to determine an ideal fibre

type for criterium riders, time trialists and road racers. For example, thanks to drafting a cyclist with a good aerobic capacity (75 ml/kg/min) and a low percentage of Type I fibres (40%) can keep pace with trained road cyclists.

Table 2.3.2: Speculates as to what may be the ideal fibre type ratios for various disciplines within the sport of cycling.

Event & performance time	Type I fibre %	Type II fibre %	VO₂max ml/kg/min
Kilometer 1000 meters: 1min 6sec	25	75	55
Criterium specialist * 50 miles: 2 hours	50	50	72
Criterium specialist ** 50 miles: 2 hours	65	35	75
Stage racer 120 miles: 4hrs, 30min	60	40	80
Time trialist 25 miles: 58 minutes	70	30	75

(Table adapted from, *Inside the cyclist: Physiology for the two-wheeled athlete*, Burke *et al.*, 1984)

* Excellent sprinter ** Poor sprinter

Work completed by Coyle *et al.*, (1992), found that besides needing a high maximal oxygen consumption for success, elite road cyclists needed to possess a higher percentage of Type I fibres and a greater amount of capillaries supplying oxygen. They also concluded that cyclists with a high percentage of Type I fibres have a distinct advantage than those with a high maximal oxygen consumption and higher percentage of Type II

fibres. These conclusions were drawn by relating muscle characteristics to a 40-kilometer time-trial performance that correlated highly with average power output during a 1-hour laboratory test. However, for shorter distances, such as a 5 km time trial, the demands for fatigue resistance, power and speed are different. No data are available on the best fibre composition for shorter distances with multiple demands.

Is a cyclist born with a certain fibre type ratio, or can it be changed with training? One purpose of training is to develop and improve the metabolic capacities of muscle fibres. There is much evidence to show alterations in metabolic characteristics of skeletal muscle fibres, for example oxidative enzyme capacity (Simoneau, 1995). Although it has been debated as to whether skeletal muscle fibre type distribution, particularly the proportion of the Type I fibres, can be altered in response to several weeks of exercise training in humans (Simoneau, 1995). Two different points of view are expressed in the literature. The first one is that the percentage of Type I fibres is genetically determined and the second is that it can be significantly modified in response to exercise training. Some studies have shown no Type I modification in response to exercise training (Basset, 1994). The problem with these studies are, that they are done on a small number of subjects. Nine out of the 12 longitudinal studies were done on 10 subjects or less. Three studies, did however show significant alterations in the proportion of type I fibres. They showed that several weeks of high intensity endurance (Howald *et al.*, 1985) or intermittent (Simoneau *et al.*, 1985) exercise training increased the proportion of Type I fibres.

The subtypes of Type II do show more consistent modification to training. With training, Type IIX fibres can take on the characteristics of Type IIA fibres. This suggests that through training these fibres can change their metabolic capacity and gain endurance capacity. These changes are not only seen by qualitative histochemical analyses but also when muscle samples are analyzed biochemically for MHC composition (Staron and Johnson, 1993). Therefore, not only can metabolic capacity be altered, but also contractile properties related to myosin ATPase activity. The increase in proportion of fibres exhibiting more than one MHC isoform is also considered as another sign of fibre

transformation in response to training (Staron and Johnson, 1993). Therefore, genetic factors, for the most part, determine your muscle fibre type. You are born with a certain percentage, but can change this a little with age or training (Simoneau and Bouchard, 1995).

The above physiological parameters can be objectively and exactly determined and can therefore be used to predict performance. An understanding of muscle metabolism, however, can also provide the cyclist with important knowledge. Even if it cannot always be quantified in a way that can predict performance, this knowledge may influence training regimen and nutritional interventions.

CHAPTER 3: The Energy Systems

“ Those who are enamoured with the practice without the science, are like a pilot who goes into a ship without a rudder or compass and never has any certainty of where he is going.”

-Leonardo Da Vinci-

The term energy systems is used to refer to a physiological entity that produces ATP to sustain cellular processes, including muscle contraction (Hawley and Hopkins, 1995). As the research project was conducted on cyclists, the question arises, how do muscles produce the energy needed to ride a bicycle? On the surface it may seem simple: breathe in oxygen and transport it to the working muscle, where it combines with food fuels and the muscles contract to produce force. But it is not that simple. This section will provide an integrated synthesis of traditional biochemistry, physiology, exercise physiology textbook knowledge, as well as the refinements and details provided by current reviews and recent experimental data.

The metabolic processes that supply the energy needs of muscle contraction usually take place in the presence of adequate O₂ to oxidize the carbohydrate sources of energy completely to CO₂ and H₂O. This constitutes aerobic muscle activity. However energy for exercise that is intense and where exhaustion sets in within one minute or less must be supplied largely by anaerobic processes, because aerobic processes cannot supply energy fast enough to supply such a demand (De Vries and Housh, 1994). Thus, the energy needed for muscular contraction is produced by three independent systems: the phosphagen, anaerobic (oxygen independent) glycolytic and aerobic (oxygen dependent) systems (Åstrand *et al.*, 1970; Gollnick, 1985). The first two systems are functions of anaerobic metabolism and the third is obviously a function of aerobic metabolism. The phosphagen system uses intracellular stores of adenosine tri-phosphate (ATP) and creatine phosphate (CP) to provide the energy needed for maximal bouts of strength or speed that last for a few seconds (Gaitanos *et al.*, 1993). For high intensity exercise of up to one minute in duration, energy is provided mostly by the anaerobic glycolytic system,

which breaks down muscle glycogen without consuming oxygen (Hawley and Hopkins, 1995). Exercise lasting longer than one minute is powered mostly by energy provided by the aerobic system, which uses oxygen in the breakdown of carbohydrates (glycogen and glucose), lipids (triglycerides, fatty acids and ketones) and to a small extent, amino acids (Hawley and Hopkins, 1995). To get a clearer understanding of how the different systems function and inter-relate, each system will be first looked at as a separate entity.

3.1 The Phosphagen system.

In this system two high-energy phosphate compounds, adenosine triphosphate (ATP) and creatine phosphate (CP) are used to store energy. During muscle contractions the stores of ATP are broken down to produce free energy and the following metabolites:



The primary source of energy for muscle contraction is ATP. The ATP molecule allows chemically bound energy to be converted to kinetic energy. The energy is released when ATP is hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate (P_i) at the crossbridges of the myofibrillar proteins known as the actomyosin complex (Geeves, 1991). The liberated energy permits the cross-bridges from the myosin molecules to slide over the thinner actin filaments. ATP is also involved in the restoration of a low intracellular calcium concentration (sarcoplasmic reticulum ATPase) and the resting membrane potential ($\text{Na}^+ \text{-K}^+$ ATPase) during relaxation before a subsequent activation of the same fibre is possible (Vandervoort *et al.*, 1983). However, the ATP stores can be depleted very quickly and therefore the compound CP is brought into play almost immediately and before ATP can be depleted. It undergoes a reversible reaction to release the following:



Therefore the high-energy phosphate from CP can combine with ADP to resynthesize ATP and muscle contraction can continue. The main reason this reaction is activated is to buffer the rapid accumulation of ADP, resulting from ATP hydrolysis in the multitude of energy requiring processes of muscle contraction and relaxation, in order to maintain an optimal ATP:ADP ratio such that contraction can continue. The momentary rise in ADP concentration is the primary stimulus to CP hydrolysis via the creatine kinase reaction. The CP reservoir is relatively large compared with that of ATP and amounts to 70-80 mmol.kg⁻¹ dry mass at rest (Spriet, 1995). Margaria *et al.*, (1969) proposed that CP degradation was the immediate and only substrate for ATP resynthesis during the early stages (< 10 sec) of intense exercise. Upon depletion of this substrate, glycogenolysis is activated to provide a continued ATP supply via glycolysis.

For many years this “serial mobilization” theory of anaerobic ATP provision was used to explain how human skeletal muscle responded to the large demand for ATP during high-intensity exercise (Spriet, 1995). Evidence since that time has shown that CP degradation is instantaneous at the onset of exercise and this was proven with biopsies taken following less than 10 seconds of sprint cycling. It was shown that CP concentration fell to approximately 50% of resting values and glycolytic intermediates increased up to 30 fold, thus rapid CP degradation was confirmed (Jones *et al.*, 1985). The CP store is thus a powerful energy buffer, as small changes in ATP and ADP concentrations ensure rapid ATP resynthesis.

In a study by Spriet *et al.*, (1987), they electrically stimulated the quadriceps femoris muscles of seven males under extended anaerobic conditions to quantitate anaerobic energy release and the contribution of the glycolytic system to total ATP production. Glycolysis was responsible for 90 % of the total ATP production. Approximately 90, 55 and 50 mmol of ATP/kg dry muscle was produced during the initial three 16-contraction periods, respectively. During the final 16 contractions only 15 mmol of ATP was regenerated through glycolysis, therefore glycolysis was highest in the first contraction.

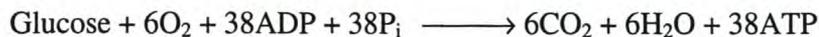
There is more experimental evidence that does not support the view that anaerobic glycolysis is only activated when CP stores are depleted in maximally contracting muscle. Findings by Jacobs *et al.*, (1983), evaluated the extent of anaerobic glycogenolysis, as indicated by intramuscular lactate concentration, after 10 and 30 seconds of supramaximal exercise. They proposed that anaerobic glycolysis commenced with the onset of muscular contraction, as lactate accumulated to 46.1 and 25.2 mmol/kg dry muscle following 10 seconds of maximal cycling in males and females respectively. Large accumulations were reported (19-61 mmol/kg dry mass) following only 6 and 10 seconds of cycling at high power outputs (Jones *et al.*, 1985). The CP degradation in this study demonstrated that CP stores had only depleted to approximately 25-33 % of the resting content, which thus suggested that CP degradation and anaerobic glycolysis were activated simultaneously at the onset of high-intensity activity.

To put the phosphagen energy system into a cycling perspective, the first few seconds of high-intensity work, e.g. intervals, sprints and racing starts will certainly be the best stimulus for this system. The quickness and explosiveness of the cyclists are also determined by other factors such as muscle-fibre type (discussed in section 3.3.1)

3.2 The Anaerobic Glycolytic System.

The other anaerobic system is the glycolytic system. Its name is derived from the pathway that can produce ATP from the utilization of carbohydrates without the use of oxygen (also known as glycolysis), in particular the glycogen reserves in the working muscle (Green, 1997). One of the end products of glycolysis is lactate. This system is responsible for most of the energy generated in maximal efforts lasting from 10-15 seconds up to 1-minute (Hawley and Hopkins, 1995).

The continuous demand for energy in this system requires that ATP be anaerobically resynthesized as follows:



For this reaction to occur successfully, glucose has to be transported into the muscle. A specific transporter protein (GLUT 4) is involved in the passage of glucose molecules across the cell membrane of muscle tissue. Once the glucose molecule is inside the cell, the first step of glycolysis is an irreversible phosphorylation to prevent loss of this valuable nutrient from the cell: glucose is converted to glucose-6-phosphate (G6-P) with the aid of the enzyme hexokinase. The hexokinase reaction is an energy consuming reaction, requiring the investment of one molecule of ATP per molecule of glucose.

There are two sources of carbohydrate substrate for this pathway, extracellular glucose and intracellular glycogen. If glycogen, rather than blood glucose, is the substrate for glycolysis, the first step is to split off a single glucose molecule. This is achieved by the enzyme glycogen phosphorylase and the products are glucose 1-phosphate (G1-P) and a glycogen molecule that is one glucose residue shorter than the original. G1-P is then rapidly converted to G6-P by the enzyme phosphoglucomutase, which then proceeds down the glycolytic pathway. There is no breakdown of ATP in this first reaction. The pathway continues and glycogenolysis and glycolysis require more input of ATP. This occurs at the step where fructose 6-phosphate (F6-P) is converted to 1,6-diphosphate (FDP) by the enzyme phosphofructokinase (PFK). PFK plays an important role in controlling flux through the pathway. So far, glycolysis, which is intended to make energy available to the cells, has required the investment of two ATP molecules, with no immediate return.

Glycolysis effectively converts a 6-carbon glucose molecule to two 3-carbon molecules, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Each of the following steps in glycolysis can thus be considered to occur in duplicate. Glyceraldehyde 3-phosphate is then converted to 1,3-diphosphoglyceric acid that receives its additional phosphate group from inorganic phosphate, so there is no further input of ATP. In the next step however, ATP is formed and 1,3-DPG is converted to 3-phosphoglycerate. The pathway continues until phosphoenolpyruvate (PEP) is formed. The last step in glycolysis

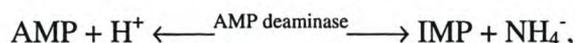
then occurs and results in the transfer of the phosphate group from PEP to ADP and the resulting formation of ATP and pyruvate. Thus the net effect of glycolysis can be seen to be the conversion of one molecule of glucose to two molecules of pyruvate and net formation of two molecules of ATP. If glycogen, rather than glucose is the starting point, net three molecules of ATP are produced. Pyruvate can now go in two directions: reduction of pyruvate to lactate in the absence of oxygen or pyruvate may undergo oxidative metabolism to CO₂ and water and thus the onset of aerobic metabolism. Thus the end product of aerobic glycolysis is pyruvate, while the end product of anaerobic glycolysis is lactate.

Lactate can accumulate within the muscle cells and reach concentrations higher than any of the glycolytic intermediates. Lactate will begin to diffuse into the extracellular space and eventually will begin to accumulate in the blood. Lactate together with hydrogen ions leave the muscle cell and has the effect of making the buffer capacity of the extracellular space available to handle some of the hydrogen ions that would cause intracellular pH to fall and impair exercise performance in at least two ways. First, an increase in the hydrogen ion concentration reduces the muscles' ability to produce ATP by inhibiting key enzymes involved in both anaerobic and aerobic production of ATP. Second, hydrogen ions compete with calcium ions for binding sites on troponin, thereby hindering the contractile process (De Vries and Housh, 1994). Muscle pH at rest is approximately 7.1 but can decrease to 6.5 or less during high-intensity exercise due to the large production of lactate (Maughan *et al.*, 1997). At pH 6.5 the contractile mechanism of muscle begins to fail and there is inhibition of some of the enzyme functions. Although this does occur, the energy made available by anaerobic glycolysis allows the performance of high-intensity exercise that would otherwise be impossible (Maughan *et al.*, 1997).

When lactate accumulates within the muscle cell it can reach high concentrations of hydrogen ions, which leads to the burning sensation that cyclists experience in their quadriceps when climbing a steep hill or going for an all out final sprint. The associated accumulation of hydrogen ions results in a low pH, which stimulates the nerve endings in

the muscle and results in the perception of pain. Lactate is discussed again in section 4.1 with references to exercise studies.

One energy system that is often overlooked, even by modern exercise physiologists, is the purine nucleotide cycle (PNC) as ADP accumulation is also a factor in fatigue (Cooke *et al.*, 1988). Exercise of a high intensity may cause large decreases in the ATP concentrations of the skeletal muscle (Cheetham *et al.*, 1986). Simultaneously, an increased production of ADP activates adenylate kinase, which together with AMP deaminase maintains the balance of the adenine nucleotide pool, the ATP:ADP ratio.



where AMP is adenosine 5'-monophosphate and IMP is inosine monophosphate.

Skeletal muscle adenine nucleotide loss can occur in two ways during exercise:

1. Deamination of AMP to IMP and ammonia catalyzed by AMP-deaminase. A large amount of IMP can be formed in this way, especially if the demand for ATP is larger than the capacity to rephosphorylate ADP
2. There is also the dephosphorylation of AMP to adenosine, catalyzed by 5'-nucleotidase.

Inosine-5'-monophosphate (IMP) and adenosine can further be degraded to inosine, xanthine and hypoxanthine.

Muscle does not possess the enzyme required to convert hypoxanthine to inosine and therefore, once formed hypoxanthine tends to leave the muscle and is eventually converted to uric acid in the liver and is eventually excreted by the kidneys. Alternatively IMP can be converted to adenylo-succinate, thus the deamination of AMP to IMP and the subsequent reamination of IMP to AMP constitute the completion of the purine

nucleotide cycle. But adenylo-succinate occurs in low concentrations after muscle fatigue, which is a sign that the adenylo-succinase activity is inhibited during exercise.

3.3The Aerobic System

As discussed earlier, the aerobic system generates ATP from the oxidative breakdown of fuels, like carbohydrates, lipids and some amino acids. Exercise lasting longer than one-minute is usually powered predominantly by the aerobic system (Hawley and Hopkins, 1995). However, it is a misconception that neither CP nor anaerobic glycolysis are any longer operative. To prove this Withers *et al.*, (1991) showed that aerobic metabolism was estimated to comprise 28, 49 and 64 % of the overall energy release for 30, 60 and 90 seconds of maximal cycling. So when high intensity exercise was extended to 90 seconds, anaerobic metabolism contributed 36-54 % of the required energy and aerobic contribution was 46-64 % (Serresse *et al.*, 1988; Withers *et al.*, 1991). This shows that CP and anaerobic glycolysis are still functioning, be it a smaller percentage than at the onset of the exercise, together with the aerobic system.

As discussed earlier glucose becomes available as an energy substrate either by glycogenolysis of glycogen stored in the muscle fiber or by the transportation of blood glucose into the muscle fibre. Glycolysis proceeds until two molecules of pyruvate are formed. In the anaerobic glycolytic system the reaction proceeds from here with the formation of lactate. In the presence of sufficient oxygen the pyruvate is able to enter the mitochondria and proceeds through a metabolic course known as the Krebs cycle (De Vries and Housh, 1994).

The Krebs cycle equation is as follows:



The two pyruvate molecules must now be converted into two molecules of acetyl coenzyme A (acetyl-CoA). Acetyl-CoA, the major fuel for the Krebs cycle in the mitochondrion, combines with the oxaloacetate to become citrate, a 6-carbon tri-carboxylic acid catalysed by the enzyme citrate synthase. Citrate is then converted to its isomer, isocitrate that undergoes further oxidative decarboxylation to α -ketoglutarate. During this reaction a molecule of CO_2 is released and the first of 3 nicotinamide adenine dinucleotide (NAD^+) in its reduced form NADH molecules are released. Another molecule of CO_2 is released and another molecule of NADH is formed as α -ketoglutarate is converted to succinyl-CoA. The energy released from the breaking of the C-S bond of succinyl-CoA is used to phosphorylate guanosine diphosphate (GDP), thus forming guanosine triphosphate (GTP) and succinate. An enzyme called nucleotide diphosphate group kinase catalyses the production of ATP from GTP. Succinate is then oxidised to fumarate and the co-enzyme flavin-adenine dinucleotide (FAD), rather than NAD^+ is reduced to FADH_2 . Fumarate goes on to be converted to malate by the enzyme fumarase. Finally oxaloacetate is formed once again from malate with the reduction of NAD^+ to NADH. In one complete cycle, 1 molecule of ATP, 3 molecules of NADH and 1 molecule of FADH_2 are formed.

Hawley and Hopkins, (1995), proposed that the oxidation of carbohydrates and lipids should be regarded as two functionally distinct aerobic power systems: the aerobic glycolytic system (which oxidizes carbohydrate for high-intensity endurance events) and the aerobic lipolytic system (which oxidizes lipids to provide a large amount of the energy for longer, less intense endurance and ultra-endurance activities). An advantage of this new view is that dietary and training regimens could be tailor made for either a high-intensity endurance activity or an ultra-endurance event.

The only difference between the two types of aerobic systems is that, in the breakdown and oxidation of lipids, a set of cytoplasmic and membrane-bound enzymes is responsible for the β -oxidation of lipids to fatty-acyl co-enzyme A (CoA), the transport of fatty-acyl CoA into the mitochondria and its conversion to acetyl-CoA. Separate sets of enzymes control the conversion of intra-and extramuscular carbohydrate to pyruvate and then to

acetyl-CoA. Thereafter the pathways for the oxidation of the acetyl moieties from either lipids or carbohydrates are the same (Hawley and Hopkins, 1995).

So what makes these two aerobic systems distinct? With regards to training they are distinctly different. For example, activities that engage the anaerobic glycolytic pathway (AnGP) also engage either phosphagen or aerobic glycolytic power systems. But activities that are short and intense (<30 sec) only use phosphagen and AnGP and do not result in maximal oxygen consumption or adaptation thereof (Jenkins *et al.*, 1994a; Linossier *et al.*, 1993).

Hargreaves *et al.*, (1991), reported no sparing of muscle glycogen in humans after one-legged knee extension exercise for 1 hour after 30 minutes of an infusion of a fat emulsion and heparin, which was also administered during exercise. In a study by Vukovich *et al.*, (1993), they wanted to determine whether saturated or unsaturated fatty acids affected muscle glycogenolysis to varying degrees in cycling exercise. Their results confirmed that increasing FFA will spare glycogen during exercise at 70 % of VO_2max on a cycle ergometer. The magnitude of the sparing was not related to the increase in the FFA above the control trial levels and the sparing appeared to be dependent on the saturation of FFA.

Brooks and Mercier, (1994), proposed the “crossover” concept. According to this concept endurance training results in muscular biochemical adaptations that enhance lipid oxidation as well as decrease the sympathetic nervous system (SNS) response to a given submaximal exercise stress. These adaptations promote lipid oxidation during mild- to moderate intensity exercise. In contrast, increases in exercise intensity are perceived to increase contraction-induced muscle glycogenolysis, change of the fibre type recruitment and increase the activity of the SNS. Substrate utilization in an individual at any point in time depends on the interaction between exercise intensity-induced responses (which increase carbohydrate utilization) and endurance training-induced responses (which promote lipid oxidation). Thus, the “crossover” concept represents a theoretical means by which one can understand the effects of exercise intensity and prior endurance training on

the balance of carbohydrate and lipid metabolism during sustained exercise. It may also explain and reconcile seemingly divergent results in the literature concerning the balance of carbohydrate and lipid metabolism during exercise (Brooks and Mercier, 1994). Thus, it can once again be said that no one energy system operates entirely by itself during exercise as is shown in the following table:

Table 3.3.1: The relationship between various cycling events and energy systems

Event	Performance Time	Phosphagen System (%)	Anaerobic System (%)	Aerobic System (%)
	<i>Hours and Min</i>			
100-mile road race	3:55-4:10	—	5	95
100K criterium	2:05-2:15	5	10	85
100K team time trial	2:10-2:20	—	15	85
25-mile time trial	0:52-0:60	—	10	90
25-mile criterium	0:50-0:60	5	15	80
	<i>Min and Sec</i>			
10-mile points race (track)	20:00-25:00	10	20	70
4000m individual pursuit	4:45-5:05	20	55	25
Kilometer	1:07-1:13	80	15	5
Match sprints	0:11-0:13	98	2	—

From: "The physiology of Cycling" by E. Burke. In *Science of Cycling* (p.2) by E.R. Burke (Ed.), 1986, Champaign, IL: Human Kinetics.

For the sprinters the most important energy source is the phosphagen system. The kilometer cyclists and the pursuiter, immediately use high-energy sources but quickly activate and obtain additional energy from the anaerobic breakdown of carbohydrates. The long-distance cyclists rely on the aerobic breakdown of carbohydrates and lipids for

energy. The energy systems are thus linked to the particular distance, time and intensity at which the cyclists perform.

3.4 The Energy Systems and Short Duration, High Intensity Cycling.

Depending on the duration and intensity of the exercise bout, some or all of the energy systems may be in operation at one time. During submaximal (steady state) exercise, ATP resynthesis can be maintained by the oxidative combustion of fat and carbohydrate stores. However, during high-intensity (non-steady state) exercise the relatively slow activation and rate of energy delivery of oxidative phosphorylation cannot always meet the energy requirements of contraction.

Non-steady state exercise can be sustained for durations in excess of 5 minutes before fatigue is evident (Maughan *et al.*, 1997). The subjects in my research project completed a number of 5km cycling time trials. Half of the 5km time trials had a pre-time trial stochastic cycling test (32 min long) training intervention that consisted of submaximal steady state workloads. These workloads were interspersed by shorter time periods at either low intensities or supramaximal intensities. Under these conditions substrate oxidation can make the most significant contribution to ATP production.

The durations of the 5km time trials were approximately 7-8 minutes. Oxidative phosphorylation can contribute as much as 80 % of the total energy yield for this cycling exercise as it is summarised in the underlying table and thus indicates the importance of substrate oxidation during high intensity exercise.

Table 3.4.1: The energy yield required for cycling.

Energy Source	Exercise Time/maximal effort						
	10sec	1min	2min	4min	10min	30min	60min
Anaerobic	85	65-70	50	30	10-15	5	2
Aerobic	15	30-35	50	70	85-90	95	98

From: "The physiology of Cycling" by E. Burke. In *Science of Cycling* (p.3) by E.R. Burke (Ed.), 1986, Champaign, IL: Human Kinetics.

Under these conditions muscle glycogen would be the principal fuel used, since muscle glucose uptake is inhibited by glucose 6-phosphate accumulation and adipose tissue lipolysis is inhibited by lactate accumulation. The rate of muscle glycogen utilization during 4 minutes of fatiguing high intensity exercise is approximately $45 \text{ mmol.kg.dm}^{-1}$ (Spriet *et al.*, 1995) which is considerably lower than the rate of approximately $160 \text{ mmol.kg.dm}^{-1}$ observed during 30 seconds of maximal sprinting (Karlsson and Saltin, 1970). This glycogen utilisation can be divided into anaerobic utilisation or full oxidation. Full oxidation of carbohydrate provides far more ATP than anaerobic breakdown, even if the quantity of glycogen utilized is less. However, even assuming that only 50 % of the glycogen in the former example is oxidised, it can be calculated that the net amount of ATP generated by glycogen oxidation will be about 2 fold greater than that generated by anaerobic glycogen degradation to lactate during 30 sec of maximal sprinting (Maughan *et al.*, 1997).

When the exercise is repeated over several bouts, as is the case with the stochastic cycling test, interspersed with short rest periods, the rates of muscle CP hydrolysis and lactate accumulation may be different in each bout compared with the first bout which was started with the subject in a completely rested state. The rates decline substantially. The progressive fall in CP utilization is likely to be related to the extent of CP resynthesis between exercise bouts i.e. if recovery is insufficient to enable complete CP resynthesis to occur, this will limit anaerobic ATP resynthesis during a subsequent bout of exercise (Maughan *et al.*, 1997).

Substrate use can be determined experimentally. The respiratory exchange ratio (RER) during steady state conditions can give a reasonable estimate of the proportions of carbohydrate and lipid being oxidised. An RER of 1.0 indicates that only carbohydrates are being oxidised, whereas an RER of 0.7 indicates that fat is the substrate being oxidised. In the exercising athlete this can be different if ventilatory compensation has already occurred following buffering of hydrogen ions. Ventilatory threshold can be reached before an RER of 1 and this will affect interpretation of RER. With the methods used in my thesis, the respiratory exchange ratio (RER) can only provide information of oxidized fuels, but not anaerobic fuel utilization. This can be determined directly from muscle biopsies before and immediately after exercise, or indirectly from the accumulation of byproducts. Muscle biopsy samples were also taken to determine the depletion of high-energy phosphates as a result of the high intensity exercise protocol. The muscle biopsies were used to determine the accumulation of intramuscular energy reserves due to in vivo dietary manipulations.

3.5 Nutritional intervention

For the successful completion of any physical task, chemical energy must be converted to mechanical energy at rates appropriate to the muscles need. As discussed in previous sections, to meet the requirements, the body has evolved in various ways to fuel itself.

During the last 150 years a large number of studies were performed to determine the utilization of different foodstuffs as energy sources during exercise and to determine nutritional effects on exercise performance. Results obtained and opinions claimed have varied through the years and can be summarized according to Hultman, (1989):

Table 3.5.1: Early studies on sources of energy used for exercise.

Studies	Result
Von Liebig (1842)	Suggested that protein was the main source of energy
Pettenkoffer and Voit (1896)	Observed that the urinary output of urea was unchanged during exercise and concluded that protein was not an important substrate during exercise.
Zuntz (1896)	Showed that both fat and carbohydrates could be utilized.
Chauveau (1896)	Maintained that carbohydrate was the sole energy source.
Krogh and Linhardt (1920) & Christensen and Hansen (1939)	Made direct estimations of carbohydrate and fat utilization during exercise by measuring RER. They also showed nutritional effects both on metabolic processes and on endurance capacity during prolonged exercise.

Later studies used radio-labeled glucose (Reichard *et al.*, 1961) which demonstrated that skeletal muscle glucose uptake increased during exercise. The reintroduction of the muscle biopsy technique in the 1960's confirmed the importance of muscle glycogen availability for exercise performance and established the theoretical basis for glycogen loading (Bergstrom *et al.*, 1967). The importance of the availability of muscle glycogen and blood glucose during prolonged exercise is demonstrated by the observation that fatigue is often associated with muscle glycogen depletion or hypoglycemia and thus reduced pyruvate levels (Sahlin *et al.*, 1990).

The role of the diet in optimizing the glycogen stores in skeletal muscle and the subsequent exercise performance was confirmed in a study by Costill, (1988). The exercise and diet regimen has been referred to as glycogen super-compensation or carbohydrate loading and consists essentially of a glycogen depleting exercise followed by 3-4 days with a high carbohydrate intake. The study by Rauch *et al.*, (1995), also compared the effects of supplementing normal diets with additional carbohydrate, in the form of potato starch. Increasing the carbohydrate intake by approximately 72 % for 3 days prior to the trial elevated the pre-exercise muscle glycogen contents, improved

power output and extended the cycling distance covered in 1-hour. They were also able to show that muscle glycogen contents were similar at the end of the 3 hour trial, indicating a greater utilization of glycogen when subjects were carbohydrate loaded, which may be responsible for their improved cycling performance.

In another study, Jenkins *et al.*, (1994), examined whether a pre-exercise consumption of glucose by subjects having adhered to a 3 day low carbohydrate diet or normal carbohydrate diet would influence supramaximal intermittent exercise performance. No changes in performance were found but, the subjects that consumed the glucose solution before the trial consumed significantly less oxygen during exercise than those who had been given the placebo solution. Thomas and colleagues (1991) looked at the advantage of carbohydrate supplementation for improved performance. They hypothesized that low glycemic index (GI) foods may confer an advantage when eaten before prolonged strenuous exercise by providing a slow release source of glucose to the blood without an accompanying insulin surge. Their findings suggested that a low GI pre-exercise meal may prolong endurance capacity, as endurance time after low GI was 20 min longer than after the intake of high glycemic index food ($P < 0.05$). The mechanism for the improved performance is less post-prandial hyperglycemia and hyperinsulinemia, lower levels of plasma lactate before and during exercise and by maintaining plasma glucose and FFA at higher levels during critical periods of exercise.

Lipid fuel sources are important energy substrates for skeletal muscle metabolism during endurance exercise. Their contribution is dependent on factors such as intensity and duration of the exercise test as well as dietary and training status. In a study by Helge *et al.*, (1998), they evaluated the effect of duration of exercise on the interaction between training and a fat-rich or carbohydrate-rich diet on endurance performance. Time to exhaustion, when exercising at the same absolute workload, was similar in fat-rich conditions and carbohydrate-rich groups and was significantly increased by 166% and 150% in fat-rich and carbohydrate-rich respectively, after 4-weeks. After 4-weeks RER was significantly lower during exercise in the fat-rich diet groups both compared with the initial test with the carbohydrate-rich diet, while no changes were seen in the

carbohydrate-rich diet group. Thus their findings showed that endurance performance was enhanced similarly after both 2 and 4-weeks of adaptation to training and a fat-rich or carbohydrate-rich diet.

Proteins and amino acids are often ignored in discussions of metabolism during exercise probably for two reasons: firstly, amino acids contribute only a minor portion between 5-10% (Brooks, 1987) of the energy consumed during exercise, although one must recognise that even a minor contribution to the energy consumption is important in conditions of high energy demands over a prolonged period of time; and secondly little is known about this complex aspect of metabolism. Although it is important to mention that the protein composition of muscle is critical to the character of the tissue, metabolism of amino groups is very dynamic and may be critical to the overall regulation of metabolism and numerous amino acid pathways directly involved in glycolytic or TCA intermediates as either substrates or products (Graham *et al.*, 1995). Thus, by incorporating amino acid metabolism, better integration of the carbohydrate and fat metabolism can come to the fore.

The ability of the skeletal muscle to utilise amino acids as an energy source has been regarded as limited but certainly not insignificant. In a study by Maclean *et al.*, (1991), it was demonstrated that during prolonged exercise (2-3 hours) there was no net accumulation of branched chain amino acids (BCAA) isoleucine, leucine and valine in the plasma or in contracting muscle. This suggests that active muscle was responsible for the removal and oxidation of any amino acids released into the plasma during exercise.

The question of what an athlete should eat in order to achieve superior performance must be one of the oldest questions in sport history. Today we have a comprehensive knowledge of essential nutrients and supplements required for maintaining good health. However the exact nutritional requirements needed to perform varies from athlete to athlete and sport to sport. As the research project was conducted on trained cyclists, the frequently asked question of how can nutrition improve cycling performance, can be asked. The main nutritional need of cyclists is an increased energy turnover. The need

becomes greater the more intensive your cycling exercise and the longer your total energy expenditure.

A study on cyclists in the Tour de France (Brouns *et al.*, 1989), looked at the effect of carbohydrate dietary manipulation during 2-days of long lasting exhausting cycling on food and fluid intake, energy balance, nitrogen balance and nutrient oxidation. They concluded that cyclists are able to maintain energy balance when energy expenditure levels exceed 20 MJ by using concentrated carbohydrate solutions in addition to normal carbohydrate-rich diet. In contrast, by using a normal carbohydrate-rich diet alone energy balance cannot be maintained in such a situation. The *ad libitum* supply of 20% and 10% carbohydrate solutions during cycling as was conducted did not impair fluid intake or overall fluid status of the body compared with water intake. The intake of substantial amounts of a carbohydrate solution during exercise causes an increased carbohydrate, a decreased fat and a decreased protein oxidation compared to normal carbohydrate diet. They finally deduced that the recommended level of protein intake for endurance athletes performing highly intensive sustained exercise, consuming a carbohydrate-rich diet and being in a positive energy balance, most probably is in the range of 1.5-1.8 g.kg⁻¹ body weight per day. Thus, nutritional choice can have an effect on performance in the range of duration simulating 2 days of the Tour de France race. However, cycling of shorter duration and higher intensity may require different nutritional intervention to optimize performance.

A growing body of evidence is becoming available to indicate that dietary creatine (Cr) maybe a necessary requirement for individuals wishing to optimize their ability to perform high-intensity exercise. The nutritional intervention in the thesis research project was that of creatine monohydrate supplementation for the active agent group and a glucose polymer substitute for the placebo group. Despite the overwhelming concentration of researchers on endurance exercise (Barnett *et al.*, 1996; Godley and Yates 1997), the advantages of creatine supplementation has shifted focus to shorter duration and higher intensity exercise (Balsom *et al.*, 1993; Kirksey *et al.*, 1997; Prevost

et al., 1997) to optimize performance. Creatine is not a new phenomenon and is discussed in great detail in Chapter 5.

Chapter 4: Muscle metabolism.

4.1 Lactate

Lactate is the by-product of anaerobic energy production. Under these conditions, glucose degradation stops at the 3-carbon lactate. The lactate so produced is released into the blood stream and picked up by the liver, which converts the lactate back to glucose. This glucose returns to the muscle for further glycolysis. Muscle lactate can also be harnessed by the cardiac muscle or other skeletal muscles working at lower intensities, that oxidizes it as a source of energy.

Can training alter blood lactate accumulation? It appears so. When the aerobic capacity of the cyclist is increased with training, less lactate is produced at a certain workload than in the untrained state at the same absolute workload (Brooks, 1986). This occurs because the Krebs cycle is able to remove lactate's precursor, pyruvate at a sufficient rate to prevent a large conversion to lactate. Despite the widespread use of blood and plasma lactate concentrations in assessing performance, interpretation and application of changes in lactate levels have shown considerable variation. The following is a table of six commonly used lactate parameters:

Table 4.1: Commonly used lactate parameters.

Researcher	Lactate Parameter
Yoshida <i>et al.</i> , 1984	Lactate threshold (LT) (the power output at which [plasma lactate] begins to increase sharply above the resting level during an incremental exercise test. Commonly used by many researchers.
Thoden, 1991	LT ₁ (the power output at which plasma lactate increases by 1mM or more compared with the previous workload.
Cheng <i>et al.</i> , 1992	LT _D (the lactate threshold calculated by the D-max method)
Cheng <i>et al.</i> , 1992	LT _{MOD} (the lactate threshold calculated by a modified D-max method)
Kindermann <i>et al.</i> , 1979	L ₄ (the power output at which plasma lactate reaches a concentration of 4 mmol.L ⁻¹)
Beaver <i>et al.</i> , 1985	LT _{LOG} {the power output at which plasma lactate concentration begins to increase when the log[La] is plotted against the log (power output)}

Irrespective of both the controversy surrounding the concept of the “lactate threshold” and of arguments over nomenclature, lactate parameters have proven useful as determinants of endurance performance. What is uncertain is which of the fore-mentioned parameters best predict 1-hour endurance performance. Bishop and colleagues (1998) concluded that every lactate parameter calculated in their study was better correlated with average power output during the 1-hour cycle than was VO₂max. Of the six parameters LT_D was the most highly correlated with one hour cycle performance ($r = 0.84, P < 0.001$).

Comparison of different studies should take into consideration differences in blood sampling methods and/or exercise protocol. The choice of blood sampling site (arterial, venous or capillary) and the choice of blood media analyzed (plasma, lysed or precipitate whole blood) have been shown to influence the exercise intensity corresponding to a fixed lactate concentration (Foxdal *et al.*, 1991; Yoshida *et al.*, 1982), but not to significantly alter lactate threshold. Lactate concentrations and lactate parameters have also been shown to be exercise protocol specific (Fry *et al.*, 1992; Heck *et al.*, 1985).

The question that still remains is why lactate parameters should provide a better predictor of endurance performance than VO_2max ? It could be that lactate parameters may be related to a rate of energy expenditure at which muscle cell homeostasis is sufficiently disturbed to stimulate glycogenolysis, resulting in accelerated glycogen utilization and lactate production. The constantly strong relationship between endurance performance and lactate parameters provides a simple method of estimating a rate of energy expenditure, which will not prematurely deplete glycogen stores (Coggan and Coyle, 1991).

The simple measure of plasma lactate concentration is affected by lactate appearance rate and lactate disappearance rate. Lactate disappearance (= removal/clearance) rate is determined by both oxidation of lactate and utilization of lactate as a gluconeogenic precursor. Reported patterns of lactate removal in humans suggest that a larger fraction of the post exercise lactate load is removed gluconeogenically under some circumstances. These circumstances were shown by Åstrand *et al.*, (1986), who estimated a theoretical maximum of 40% for lactate oxidation following brief intense exercise, whereas replenishment of muscle glycogen was proposed as the fate of 50% of the post-exercise lactate pool. Lactate removal across the splanchnic bed was estimated as only 10% of the total. In contrast Bangsbo *et al.*, (1991), estimated that only 13-27% of the lactate removed in humans recovering from 3 minutes of intense leg exercise served as a gluconeogenic substrate. Lactate irreversible disposal and oxidation rates were also studied by Mazzeo *et al.*, (1986), on subjects at rest, during easy exercise and during hard

exercise. They concluded that lactate disposal rate is directly related to the metabolic rate, oxidation is the major fate of lactate removal during exercise and that blood lactate concentration is not an accurate indicator of lactate disposal and oxidation.

Bergman *et al.*, (1999), evaluated the hypothesis that endurance training decreases arterial lactate concentration during continuous exercise by decreasing net lactate release and appearance rates and increasing metabolic clearance rate. They concluded that active skeletal muscle is not solely responsible for elevated arterial lactate concentration and training increases leg lactate clearance, decreases whole body and leg lactate production at a given moderate-intensity power output and increases both whole body and leg lactate clearance at a high relative power output.

A second important finding in the study by Bishop and colleagues (1998) was the strong relationship between peak sustained power output (PSPO) and the one hour cycle performance ($r = 0.81$, $P < 0.001$). This is in agreement with previous research on cyclists (Hawley and Noakes, 1992). It shows that PSPO is a better predictor of endurance performance than $VO_2\text{max}$ and similar to LT_{MOD} (the lactate threshold calculated by a modified D-max method). Thus cyclists who record both a high PSPO and have delayed accumulation of lactate are likely to perform best. A question that remains to be answered is the relative importance of PSPO vs. lactate parameters on performance in even shorter, high intensity endurance events.

4.2 The Purine Nucleotide cycle

As indicated in section 2.2, it is suggested that adenine nucleotide loss occurs during intense muscle contraction. Intense exercise to fatigue can lead to a 20% decrease in ATP concentrations determined immediately after exercise. Of this decrease 65% can be appointed to IMP formation, 14% to ADP and AMP increase and 2% to the formation of inosine, that means that 18% of the purine that was in the cell before exercise, can not be accounted for (Goodman and Lowenstein, 1977). This implies that it has left the muscle

compartment in one or another form and is likely to be found in the form of one or more metabolites in the blood compartment. During the initial stages of recovery the IMP concentration decreases while [ammonia] remains constant. This decrease is due to the reactions that lead to the formation of xanthine.

As discussed previously, the purines must move out of the muscle to account for the decrease in purine levels in the muscle cell. During exercise the [hypoxanthine] increases slightly in the blood. During the recovery phase it can increase 18-fold, with a peak reached approximately 15 minutes after fatigue (Hellsten Westing *et al.*, 1989). The increase in plasma inosine levels is similar, but the peak concentration is reached approximately 20-minutes after fatigue and then decreases sharply (Hellsten Westing *et al.*, 1989).

The appearance of hypoxanthine and inosine in the blood plasma is paired directly with a net uptake of these molecules in the liver. This uptake takes place by means of diffusion or facilitated diffusion. The appearance of hypoxanthine and inosine is also paired with the release of urate and xanthine into the blood stream. This is an indication that these two purines in the liver are irreversibly converted to urate and xanthine and thus making the liver one of the main areas of urate synthesis. Inosine and hypoxanthine can however be changed to IMP and AMP in the liver (Hellsten Westing *et al.*, 1989), so not all is converted and released as urate.

Skeletal muscle produces ammonia during short-term, intense exercise as well as prolonged, sub-maximal exercise. It has already been suggested that ammonia produced during both types of exercise originates from AMP-deaminase reaction of the nucleotide cycle. However, amino acid catabolism can also produce ammonia and the oxidation of branched chain amino acids (BCAA), isoleucine, leucine and valine increases in skeletal muscle during long duration exercise (Hargreaves, 1991). During exercise of approximately 40% VO_2max and greater, ammonia production increases proportionally to intensity. At lower exercise intensities, the muscle ammonia release tends to balance the clearance and little change in plasma ammonia is found. However, plasma and muscle

ammonia concentrations rise rapidly during more intensive exercise because clearance can not match ammonia release from active muscle. In healthy subjects, values of 100-150 μM are typically observed at exhaustion after long duration exercise (Graham and Mclean, 1992).

CHAPTER 5: Creatine

5.1 Historical Background

Despite the fact that athletes have only used creatine as an ergogenic aid for about a decade, the “creatine time line” dates back a long way. The French scientist Michel-Eugene Chevreul, who named it after the Greek word for flesh, discovered creatine (Cr) in meat extracts in 1832. Due to detection problems, it was not until 1847 that Liebig was able to confirm the presence of creatine as a regular constituent of meat (reviewed by Demant and Rhodes, 1999). At this time Liebig also observed that the meat of wild foxes that were killed in the chase had ten times the amount of creatine than those in captivity and came to the conclusion that working of the muscle resulted in the increase and accumulation of creatine. It was also at this time that two other researchers, Heintz and Pettenkoffer, also discovered a substance in urine, creatinine (a byproduct of creatine degradation), confirmed later by Liebig (reviewed by Balsom *et al.*, 1994). The concentration of creatinine in urine was directly related to muscle mass and therefore it was speculated that the creatinine found in urine was derived directly from the creatine stored in muscles.

Creatine research continued, but it was not until the beginning of the 20th century that it was observed that not all the creatine that was ingested by animals and humans was reclaimed in the urine, which suggested that the body was retaining some. In 1912 and 1914, Denis and Folin determined that the creatine content of cat muscle increased by 70% following creatine ingestion (reviewed by Demant and Rhodes, 1999). By 1923 Hahn and Meyer had estimated the total creatine content of a 70kg male to be around 140g (Needham, 1971). Thus, after only seven days of supplementation one could have ingested an equal amount to the total body store.

In 1927 and 1929, Fiske and Subbarow discovered a labile phosphorous in the resting muscle of cat, which they named phosphocreatine or creatine phosphate (CP) (reviewed by Balsom *et al.*, 1994). It was shown that the concentrations of CP decreased for a period of time during electrical stimulation of skeletal muscle, but then increased to previous levels following a recovery period (Hunter, 1928). Due to the early work of these authors, the identification of free creatine (Cr_{free}) and CP forms have been recognised as key intermediates of skeletal muscle metabolism. It is surprising, therefore, that attempts to supplement in athletes took so many more decades to come about.

Through the re-introduction of the needle biopsy technique (Bergstrom, 1962) it was possible to study the breakdown and resynthesis of adenosine triphosphate (ATP) and CP in skeletal muscle, as well as to determine the role CP plays in skeletal muscle metabolism during exercise (Hultman *et al.*, 1967). More recently the role of Cr_{phos} in skeletal muscle metabolism has been studied with nuclear magnetic resonance spectroscopy (NMR) techniques (Kreis *et al.*, 1999). Although studies with creatine supplementation can be traced back to the end of the 19th century, it appears that only recently has the influence of creatine supplementation on exercise performance in humans been studied (reviewed by Balsom, *et al.*, 1995).

It appears that the first documented use of Cr supplementation was with the British athletes training for the 1992 Olympics in Barcelona. Creatine was credited with powering several of the British athletes to gold medals. The London Times (August 7, 1992) reported that Linford Christie, 100-meter gold medalist, trained with creatine before the 1992 Olympics, it also reported that Colin Jackson, the champion British 110 meter hurdler, began taking creatine just before the Olympics. Although he did not win the gold medal at the Olympics, he soon beat the Olympic gold medalist, Mark McCoy, on several occasions. Shortly thereafter, U.S. champion athletes began using creatine (Passwater, 1997). This indicates that the athletes were possibly ahead of the scientists in the “creatine game”.

Articles in athletic magazines occasionally mention a rumour that USSR and Bulgarian athletes may have been using creatine for many years, perhaps since the 1970's. No one in the creatine field of research has found any documentation on this use.

Creatine supplements especially designed for performance and strength enhancement were not commercially available until about 1993. In 1993, researchers Almada and Byrd introduced their formulation based on reports in the scientific literature, plus their own research. In late 1992 and early 1993, the results seemed so unbelievable that they had little success convincing established companies to introduce Cr supplements in a convenient form, which athletes could use to achieve Cr loading and maintenance. Thus they formed their own company, which became incorporated in mid-1993 and introduced the first commercial product especially designed to take advantage of their scientific research. Since that time, nearly all the companies making sports nutrition supplements have introduced kindred products (Passwater, 1997). This has thus made creatine widely used and available and we can now scientifically attempt to determine who should be using it and how as well as who is using it and wasting it.

5.2 Biosynthesis

5.2.1 Biochemistry

What is creatine? Creatine is an amino acid compound that can be endogenously synthesized and creatine phosphate is an important reservoir of high-energy phosphate groups in our muscles. Its chemical name is methyl guanidine-acetic acid. The structure of creatine is



The synthesis of creatine (as reviewed by Walker, 1979) involves 3 amino acids: glycine, arginine and methionine. In the first step of biosynthesis, a portion of the amino acid arginine is removed and added to the amino acid glycine to form a new compound called

guanidinoacetic (GAA). The portion removed from arginine and transferred to glycine is called an amidine group and its transfer is made possible by the enzyme glycine transamidinase.

The second step involves removing a portion of a sulphur-containing compound called S-adenosylmethionine (SAM). SAM is derived from the amino acid methionine. The portion transferred from SAM is called a methyl group and its transfer to GAA is made possible by the enzyme guanidinoacetate methyltransferase. After the methyl group has been added to GAA, the resulting compound is called methyl guanidineacetic acid or simply creatine. So it can be said that creatine is formed from parts of the 3 amino acids – arginine, glycine and methionine - and it can also be said that they are precursors of creatine (Walker, 1979).

One could thus ask if diets sufficient in arginine, glycine and methionine be good enough supplements? Methionine is a dietary essential. It has many functions in the body and the metabolic consequences of methionine deficiency are many and varied. Methionine has a high intracellular turnover because it is involved in the initiation of peptide synthesis and in a variety of trans-methylation and trans-sulfuration reactions (Storch *et al.*, 1990). Methionine may be the endogenous amino acid that is most limiting for maintenance of body protein and nitrogen balance and for effective reutilization of other amino acids (Storch *et al.*, 1990). Since methionine is a nutritionally indispensable amino acid, its continued availability in free amino acid tissue pools is important for maintaining an anabolic drive for protein synthesis (Millward and Rivers, 1989) under conditions of greater turnover and thus essential for the formation of creatine.

Arginine is normally a nonessential amino acids in humans, but is considered essential under certain conditions. Studies have pointed to the essential nature of arginine in a variety of catabolic conditions, including surgery and trauma and in conditions where growth is accelerated (Zieve, 1986; Barbul, 1986). As arginine is a precursor of creatine phosphate, one study has shown that muscle creatine content has significantly increased by arginine supplementation (along with glycine) (Minuskin *et al.*, 1981). In this study

supplementing a 25% casein-based diet with arginine and glycine significantly improved apparent nitrogen retention both in untraumatized and traumatized rats. There is also other direct evidence that oral supplementation of the amino acid arginine leads to enhanced muscle mass and strength in conjunction with resistance training (Elam *et al.*, 1989).

The last amino acid in the trio is glycine that is the simplest of the amino acid. While being a nonessential amino acid, during periods of rapid growth the demand of glycine may be increased. As mentioned previously as well as in other studies, glycine used together with arginine has been shown to increase endogenous creatine levels (Minuskin *et al.*, 1981; Bucci, 1993). Another important aspect of glycine is that it is an important constituent of collagen. Nearly one third of amino acid residues in collagen are glycine. Collagen, an extracellular protein that is organized into soluble fibers of great tensile strength, occurs in all animals and is the most abundant protein of vertebrates. It is the major stress-bearing component of connective tissues such as bone, teeth, cartilage, skin and blood vessels (Di Pasquale, 1997).

Thus all three amino acids play a vital role on their own but to mimic the role of creatine supplementation they have to combine with one or more of the three.

5.2.2 De novo synthesis.

In humans, the enzymes involved in the *de novo* synthesis of creatine are located in the liver, pancreas and kidneys. This means that the creatine is produced outside the muscle and transported to the muscle via the vascular system. Transamidation occurs in the kidney between arginine and glycine. Methylation of guanidinoacetic acid by S-adenosylmethionine, that forms creatine, occurs in the liver. The creatine is then transported to the muscle. The normal concentration of creatine in plasma is 50 to 100 $\mu\text{mol/L}$ (Balsom *et al.*, 1994). It could thus be that due to the complexity of different places where it is made and the fact that it is actually transported in low concentrations

argues for creatine supplementation working better than the dietary intake of the precursors.

5.2.3 Creatine transporters and uptake

The structural and functional characteristics of the transporter responsible for Cr uptake from the circulation into muscle occurs by creatine entering a number of cell types by a Na-dependent transporter family. It is related to the taurine transporter and the members of the sub-family of GABA/betaine transporters (Bennet et al., 1994; Guimbal and Kilimann, 1993). The results of an unpublished study (presented May 30, 1997 at the American College of Sports Medicine Annual Meeting) indicated that ingestion of creatine with glucose, taurine and electrolytes during training promotes greater gains in upper extremity lifting volume and sprint capacity as compared to glucose, taurine and electrolyte ingestion alone (Di Pasquale, 1997).

In a study by Guerrero-Ontiveros and Walliman (1998), they presented evidence for the existence of two highly related creatine transporter (Crea T) isoforms, which are co-expressed in all tissues where the CreaT and incidentally also CK, is found, except for the liver, which according to their results also contains Crea T but no or very little CK. They also showed that both these highly homologous proteins were down regulated after chronic administration of external Cr and that this down-regulation is prevented or even counteracted by 3-guanidinopropionate (GPA), the creatine analogue.

Several lines of evidence support the hypothesis that more than one Crea T exist. Two Crea T mRNA species of 4.0-4.3 and 2.2-3.0 kb, whose tissue expression patterns differ from one another, were detected by Northern blot analysis (Gonzalez and Uhl, 1994). Barnwell *et al.*, (1995) cloned and sequenced two human cDNAs, one Crea T1, homologous throughout its length with the rat Crea T sequence and a second Crea T2, encoding a new protein and containing some regions of perfect homology with the Crea T amino acid sequence, but four segments of unique sequences. Although they did not present any evidence, they did suggest that Crea T2 mRNA might be transcribed from the Crea T1 gene, possibly by alternative splicing (Barnwell *et al.*, 1995). Thus from the

above findings there is evidence to support the existence of two different Crea T mRNA species, as well as of two Crea T transporter polypeptides with identical N- and C-termini, as judged by anti-peptide-specific antibodies. The two mRNA also correspond to the 70 and 55 kDa proteins detected by both the anti-N- as well as anti-C- terminal anti-peptide antibodies. The differential splicing, therefore must take place in a region of the mRNA coding for the amino acid sequence between the N- and C- termini (Guerrero-Ontiveros and Walliman, 1998).

The Cr analogue, 3-guanidinopropionate (GPA), competitively inhibits Crea T activity. Long term feeding (6-10 weeks) of rats with GPA results in a marked decrease in CP, Cr and ATP levels in skeletal muscle (Wyss and Walliman, 1994). On the other hand, Cr supplementation augments Cr uptake and accumulation, thus depending on the dose and type of substrate used, as well as the time of ingestion, different metabolic adaptations will occur (Guerrero-Ontiveros and Walliman, 1998). Concerning the Crea T, it can be that extra-cellular Cr may down-regulate the level of the Crea T expression and its activity in rat skeletal muscle cell line (Loike et al., 1988).

What happens with human athletes who ingest Cr? Human muscle appears to have an upper limit for its Cr content of 150-160 mmol/kg dry muscle (as reviewed by Passwater, 1997). This suggests that long term Cr intake influences the synthesis of the Crea T in order to prevent the accumulation of excessive intra-muscular Cr. The down-regulation could also be a possible side effect of Cr supplementation (discussed later). It is inadvisable to consume Cr continuously for longer period of times (more than 3 months) or to abuse Cr ingestion in order to improve performance at any cost. A creatine-free period of one month, after 3 months of creatine ingestion would be advisable (Guerrero-Ontiveros and Walliman, 1998), based on a wash-out time study (Hultman et al., 1996).

Since the functional and structural characteristics of the Cr transporter in muscle has already been described above, it may be possible to achieve higher levels of Cr in the muscle by combining other substances with Cr to enhance its uptake into the muscle. By having the optimal schedule of dosages and timing of Cr administration to maximize

storage in the muscle, it is important to remember that the level of expression of the Cr transporter is regulated by the extracellular Cr concentration as was mentioned above. Thus combining other nutritional ergogenic aids that may promote a synergistic effect with Cr should be mentioned. In a study by Green *et al.*, (1996), they investigated the effect of carbohydrate (CHO) ingestion on skeletal muscle creatine accumulation during creatine supplementation in humans. They concluded that creatine supplementation had no effect on serum insulin concentration, but creatine and CHO ingestion dramatically elevated insulin concentration ($P < 0.001$). Thus the study demonstrated that skeletal muscle creatine accumulation could be substantially augmented in humans when creatine was ingested with CHO, a response that appeared to be insulin mediated and which could possibly have important implications on maximal exercise performance.

5.3 Creatine in skeletal muscle

5.3.1 Distribution in the body and daily turnover

Creatine is found naturally in skeletal muscle, heart, brain, testes, retina and other tissues (reviewed by Wallimann and Hemmer 1994). However, virtually all (95-98 percent) of the body's creatine is stored in skeletal muscles. An average-sized healthy male may have about 120 grams of creatine stored in his body. The total creatine (Cr_{tot}) pool in humans refers to the combined amount of creatine in its free (Cr_{free}) and phosphorylated (CP) form. In skeletal muscle, approximately one quarter exists as Cr_{free} and three quarters as CP. Creatine is synthesized at a rate of 1-2 g/day (Walker, 1979). In the absence of exogenous creatine, the rate of Cr and CP being degraded to creatinine via a non-enzymatic, irreversible reaction is estimated at approximately 1.6% per day (Crim *et al.*, 1976). The creatinine is filtered in the kidneys and is excreted in the urine. Urine concentration of creatinine averages about one-tenth that of urea (Passwater, 1997). An additional 1-2 g/day of creatine is obtained in the diet, mainly from fish and meats (as reviewed by Balsom, 1994). Therefore an average-sized man with a total creatine pool of approximately 120g has a turnover rate of approximately 2g per day, as the lost creatine is replaced by both endogenous and exogenous sources. It was long believed that the endogenous synthesis is to some degree regulated by exogenous intake, likely a feedback

mechanism (Walker, 1960). The data on transporter down-regulation discussed earlier confirms this early hypothesis.

The richest food source of creatine is animal muscle such as that found in meats and fish and trace amounts found in some plants. Vegetarians have little creatine in their diets, thus vegetarians, on a creatine free diet, rely completely on endogenous synthesis (Balsom *et al.*, 1994).

To enhance sports performance, creatine supplements are usually taken in 5-gram doses, one to four times a day, as will be discussed later, depending on whether the athlete is in the loading phase or the maintenance phase. To obtain 5 grams of creatine from steak would require about 1.1 kg of fresh uncooked meat. This thus strengthens the argument of rather supplementing than increasing creatine intake in the diet.

Table 5.3.1: Creatine in selected uncooked food items. (Adapted from Balsom *et al.*, 1994)

FOOD	AMOUNT of CREATINE (g/kg)
Beef	4.5
Cod	3
Cranberries	0.02
Herring	6.5-10
Milk	0.1
Pork	5
Salmon	4.5
Tuna	4

5.3.2 Gender and age

There are very few studies to date that can be found in the literature that compare the levels of Cr_{free} and CP in the skeletal muscle of males and females (Forsberg *et al.*, 1991; Rehunen *et al.*, 1980). Forsberg *et al.*, (1991), however did find that females have slightly

higher values of Cr_{tot} in relation to tissue mass, but there has been no further evidence with regard to total creatine content to suggest whether or not a difference actually exists between males and females.

How aging can have an effect on Cr_{tot} was studied by Moller *et al.*, (1980), who deduced that there was no significant difference between the Cr_{tot} levels of young (18-36 yrs) and elderly (52-79 yrs) individuals. But he did find that the level of Cr_{free} was higher and CP was lower in the elderly individuals (Moeller *et al.*, 1980). This could imply that there might be a difference in the CK equilibrium constant.

5.3.3 Creatine and muscle fiber types

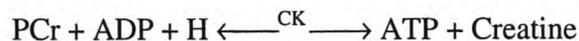
Separation of type I and type II fibers from human freeze dried muscle biopsy samples, showed that type II (fast twitch) fibers of human skeletal muscle sampled in the resting state, had a higher content of CP than type I (slow twitch) fibers (Söderlund *et al.*, 1992; Tesch *et al.*, 1989). These data support other studies showing higher levels of CP in fast twitch than in slow twitch muscle fibers in a resting state or similarly vastus lateralis vs. soleus muscle in humans (Essen, 1978; Edström *et al.*, 1992).

CP levels were also different between fiber types after exercise. During 30 seconds of maximal exercise the CP levels in type II fibers dropped below those of type I fibers. After a recovery period of 60 seconds the CP levels of the type I muscle fibers were still considerably higher than those of the type II fibers, thus type I fibers have a greater ability to resynthesize CP during recovery (Tesch *et al.*, 1989). The fact that type I muscle fibers have a greater capillary density and higher activities of oxidative enzymes may help with recovery of CP. The implication for supplementation is that type II fibers would benefit more.

5.4 Creatine Metabolism

5.4.1 Creatine and Creatine Phosphate as Temporal Energy Buffer

During intense muscle contraction, usually a physical activity of a short and intense duration, the energy required is provided by the high-energy phosphates, ATP and CP, found in the muscle (McArdle *et al.*, 1991). Immediate regeneration of ATP is needed if muscle contraction is to continue. For the first few seconds, the ATP regeneration is dependent on the re-phosphorylation of ADP from the breakdown of CP, that supplies the high-energy phosphate required for quick resynthesis of ATP from ADP, which is accomplished rapidly under anaerobic conditions via the following pathways.



The reaction shifts to the right when there is muscle contraction and ATP is hydrolysed to ADP and shifts to the left by the removal of ADP at energy generating sites (Demant and Rhodes, 1999).

The creatine kinase (CK) enzyme controls the reaction; and

Glucose/glycogen + ADP \leftrightarrow ATP + lactate (anaerobic glycolysis)

5.4.2 Creatine/Creatine Phosphate as a Spatial Energy Buffer: The Phosphocreatine Energy Shuttle

In this concept, CP is postulated to act as an energy carrier, transporting energy from the mitochondria to different ATPase sites in the cytosol. Bessman formally proposed this diffusion process in 1972, referring to it as the “phosphorylcreatine shuttle” (Bessman and Geiger, 1981). This CP energy shuttle involves 3 areas: (1) a peripheral terminus located outside the utilization site, with regards to muscle is the myosin heads; (2) an energy generating terminus which is located at the mitochondria and (3) a transferable space between the areas of production and utilization (Volek *et al.*, 1996).

When the CP molecule is broken down by one of the isoforms of creatine kinase (CK), providing a high energy phosphate for ATP resynthesis, there is the diffusion of Cr_{free} towards the mitochondrial membrane. At the membrane, the creatine is phosphorylated via another of the CK isoforms, using the high-energy phosphate from the breakdown of ATP to ADP, ATPase, in the mitochondrial membrane. The newly re-synthesized CP diffuses back to the myosin heads where its energy is once again used for ATP re-synthesis (Bessman and Geiger, 1981). The net bout of exercise can now occur (usually anaerobic). This shuttle system may also be important during aerobic exercise or in the facilitation of recovery after exercise. Thus, in normal subjects, the CP shuttle acts as a “spatial energy buffer”, providing for more efficient energy transport between sites of ATP synthesis and utilization (Bessman and Geiger, 1981).

The onset of lactate accumulation is a direct result of an increase in glycolytic flux, which is needed to meet the increased energy demands that occur during maximal exercise. At this time the muscle CP content also decreases, due to a need to rapidly re-synthesize ATP. It has been reported that glycolysis may be stimulated by a decline in CP levels, leading researchers to suggest that phosphofructokinase (PFK), a key glycolytic enzyme, is at least partly inhibited by CP. Therefore, during intense physical activity CP decreases, PFK becomes less inhibited and the rate of glycolysis increases, producing more ATP for active muscle (Demant and Rhodes, 1999).

5.5 Creatine Supplementation

5.5.1 Dosing and the effects on muscle [TCr] and [PCr]

For creatine supplementation to be effective, it must increase the amount of Cr_{tot} or CP within the muscle and these increased stores must help rapidly replenish ATP during exercise. Many supplementation strategies have been used in various attempts to increase [Cr_{tot}] and especially [CP]. The typical creatine supplementation protocol begins with a loading dose of 20 grams/day of creatine monohydrate for 5-7 days, followed by a “maintenance” dose of 2 grams/day (Hultman *et al.*, 1996), although some studies used lower doses or supplemented for fewer days. The following table lists successful creatine

supplementation studies to check the extent of increase in muscular creatine and/or creatine phosphate levels.

TABLE 5.5.1: A table of some successful creatine supplementation studies that used muscle biopsies to check the extent of increase in muscular creatine and/or creatine phosphate levels.

Investigator	Year	Dosage	Days	Mean % increase in [Cr _{tot}]	Result
Harris <i>et al.</i>	1992	20-30g	2-10	20-40%	There was a significant increase in [Cr _{tot}], of which 20-40% of the increase of [Cr _{tot}] was accounted for by [CP]. The muscle uptake of oral Cr was greatest in the first 2 days (32%), with smaller increases in subsequent days. About 20% of the creatine increase was due to increased CP levels.
Casey <i>et al.</i>	1996	20g	5	18%	There was also an increase [CP] of about 10% following supplementation
Hultman <i>et al.</i>	1996	20g or 3g	6 or 28	20%	This was a rapid protocol of 6 days together with a slow protocol of 28 days. The elevated [Cr _{tot}] was maintained when supplementation was continued at 2g/day. Although [CP] increased, the increase was not significant in each separate group studied, but was significant when the results of the groups were combined.
Greenhaff <i>et al.</i>	1994a	20g	5	25%	No statistical significant increase in [CP]. However there was 35% increase in PCr resynthesis after muscle contraction. These results led researchers to propose that there were responders and non-responders to creatine supplementation.

There were however studies that did not show statistically significant increases in creatine. An example was one study in which subjects used only 2 grams of creatine

monohydrate/day for 6 weeks and showed no beneficial effects on either muscle Cr_{tot} or CP levels (Thompson *et al.*, 1996).

Some studies have highlighted individual differences in response to creatine supplementation. In Greenhaff *et al.*, (1994b), they noted that the individuals that had the greatest increase in muscle $[Cr_{tot}]$, were mostly those who had subnormal levels before supplementation. Subnormal levels are 120 mmol creatine/kg dry matter or less. Individuals that have higher levels of muscle creatine are less responsive to creatine supplementation (Melvin *et al.*, 1998). But other factors also influence the efficacy of Cr loading.

A study by Green *et al.*, (1996), found that addition of a carbohydrate solution (90 grams, 4 times daily in solution with creatine during the loading phase) further enhanced the increase in muscle creatine concentration relative to taking creatine alone. Based on this study, the combination of creatine with sports drinks has become popular. Skeletal muscle also has a maximal creatine storage capacity of 150-160 mmol/kg, which makes over-supplementation futile (Casey *et al.*, 1996). Any excess creatine ingested will not further increase muscle creatine but will simply increase urinary creatine and creatinine excretion.

Muscle concentrations of Cr and CP, as well as urinary creatine and creatinine concentrations, return to baseline levels approximately 28 days after discontinuing creatine supplementation (Hultman *et al.*, 1996). Because of individual variability, the “wash out” time in future cross over studies should be at least 5 weeks (recommended by Juhn and Tarnopolsky, 1998).

5.5.2 Caffeine and creatine supplementation

As creatine uptake into the muscle is dependent on extracellular Na^+ , thus adrenergic stimulation of the sarcolemma might enhance muscle creatine uptake via the increased activity of the Na^+ , K^+ ATPase pump (Vanderberghe *et al.*, 1996). Therefore caffeine consumption has been hypothesized to improve the efficacy of creatine supplementation.

In Vanderberghe *et al.*, (1996), they looked at and compared creatine uptake, isometric force and isokinetic knee-extension torque production in nine healthy males following 6 days of either creatine supplementation (0.5 g/kg/day) or creatine (0.5 g/kg/day) in combination with caffeine (5 g/kg/day). There was a 3-week washout period between treatments. The results were that [CP] increased after both treatments and torque only increased after Cr supplementation without caffeine. Thus a conclusion was reached that caffeine eliminates the ergogenic effect of creatine. However criticism could be raised about the short wash out period.

Since both creatine and caffeine may facilitate human performance, another study was also conducted to assess the effects of creatine and caffeine alone and in combination, on short term maximal performance and endurance performance in trained athletes (Vanakoski *et al.*, 1998). The results were that creatine had no beneficial effects on maximal anaerobic performance or on short-term recovery from it in trained athletes. Likewise, caffeine and the combination of creatine and caffeine failed to produce any beneficial effects on anaerobic or aerobic endurance performance (Vanakoski *et al.*, 1998).

5.5.3 The effects of creatine on body composition.

Creatine supplementation results in weight gain, from 0.5 to 1.6 kg during a 5 day loading phase (Greenhaff *et al.*, 1994; Dawson *et al.*, 1995; Barnett *et al.*, 1996) and even more with prolonged use (Vanderberghe *et al.*, 1997). The weight gain is initially due to water retention (Vanderberghe *et al.*, 1997) as indicated by decreased urinary volume during this time.

When combined with physical training, Cr supplementation may lead to an increase in lean body mass. A study on male and female track athletes reported that there was an increase in lean body mass of up to 4.8 kg, as estimated by skin-fold measurements following 6 weeks of creatine supplementation (20 grams a day), (Kirksey *et al.*, 1997).

Findings like these suggest that Cr supplementation can lead to an increase in lean body mass, but how it exactly works or what causes it is still not completely understood, it could be water retention or protein synthesis. More research is still needed.

5.5.4 Potential side effects of creatine supplementation

Adverse effects of oral creatine have not been extensively studied, though concerns have been prevalent in the media. Thus far it seems that creatine is safe, there have been no adverse effects reported except some gastric upset. Muscle cramping is a common report from athletic trainers. Since water retention occurs with creatine supplementation (Hultman *et al.*, 1996), it is speculated that this effect increases skeletal compartment pressure, leading to the risk of muscle dysfunction. In some studies that have monitored performance (Vandenberge *et al.*, 1997; Kreider *et al.*, 1998) none of the subjects experienced muscle cramping. However these studies had sample sizes of 25 or less which is sub-optimal for comprehensive statistical analyses of side effects.

Diarrhea and gastrointestinal pain have also been reported anecdotally. Once again performance studies reported none of these symptoms and again the samples were too small. It can be understandable that 20 grams a day for 5 days can be excessive for some digestive systems as the average diet only includes 1-2 grams a day.

Two published case reports of renal dysfunction in subjects taking creatine have raised some concerns (Pritchard *et al.*, 1998; Koshy *et al.*, 1999). Short-term (5 day) creatine supplementation does not appear to impair function in the healthy kidney. Supplementation can increase urinary creatine excretion rate 90-fold, long term adverse affects are unclear (Vandenberge *et al.*, 1997). The only advice is that people with pre-existing renal disease and those with a potential for renal dysfunction (diabetes) should not use creatine supplementation. Other unstudied areas of concern regarding effects of Cr supplementation are summarised in the table below:

Table 5.5.4: Concerns Regarding the Effects of Oral Creatine Supplementation

Cancer risk	Cr and CP –creatine kinase system may be involved in cellular oncogenesis (Bergnes <i>et al.</i> 1996). This can only be deduced with long term studies and then it can be seen whether it is beneficial, detrimental or no effect.
Cardiovascular system	The effect of long-term oral creatine on cardiac muscle creatine concentration and cardiac function is unknown (Mihk <i>et al.</i> , 1998). In vitro studies have shown that creatine may play a role in the development of cardiac muscle hypertrophy by stimulating protein synthesis (Ingwall, 1975). There has been no effect with short-term use (Mihk <i>et al.</i> , 1998).
Children and adolescents	Theoretical concerns focus on the extra load imposed on developing kidneys and other organs and the effects of creatine on muscle bone junctions in the skeletally immature. Because the children and adolescent population is not physiologically analogous to an adult population, studies specific to the 18-and-under age group are necessary before justifying creatine supplementation in this select group.
Fluid balance	Intracellular fluid retention in muscle may predispose creatine users to dehydration, but studies are lacking. However most creatine manufacturers recommend proper hydration.
Liver	Studies of up to 8 weeks of supplementation show minimal or no liver enzyme increase (Kreider <i>et al.</i> , 1998). It is not known if the suppression of endogenous creatine synthesis with long-term use is reversible.
Nervous system	Creatine is found naturally in the brain tissue (Vandenberge <i>et al.</i> , 1997). The true effect of Cr supplementation on the brain is unknown and further investigation is needed.
Reproductive system	Creatine is found in the testes and the sertoli cells have been identified as a site of creatine synthesis and Cr and CP are involved in sperm metabolism (Juhn <i>et al.</i> , 1998). The effects of Cr supplementation have not been studied and the possible concern is the reversibility of the suppression of endogenous synthesis.

Previous table adapted from: Juhn MS, Tarnopolsky M. Potential side effects of oral creatine supplementation: A critical review. Clin J Sports Med 1998; 8(4): 298-304.

5.5.5 Ethics and Legality

Creatine is not a steroid or a drug. Its use as a performance enhancer has not been banned by the International Olympic Committee. Creatine supplementation is a practice similar to carbohydrate loading, which is accepted. Since it is present in everyone's blood and everyone excretes some creatine in the urine, there would be no practical way to test for creatine supplementation if it were banned. The decision to use creatine as a means to enhance sport performance is left to the discretion of the individual athlete.

5.6 Creatine and Exercise Performance.

5.6.1 High intensity, short term exercise

Researchers first investigated the ergogenic effects of short-term creatine loading. A typical study usually consisted of the popular protocol of 5 grams four times a day for 5-7 days. Virtually all studies also had placebo groups that were given a glucose polymer. Supplementation was usually done in a double blind manner, meaning that neither the subject nor researcher knew what was being supplemented until the tests were complete. Due to the fact that the research project in this thesis was conducted on cyclists, this literature review will focus on the role of creatine on exercise performance in cycling. For a summary see tables 9a,b and c.

In a study of short term, high intensity (< 30 sec) cycle ergometer exercise performance tests, Balsom *et al.*, (1993a), assigned 16 male subjects to a placebo or creatine supplement group (25 g/day). The subjects had to undergo two such tests before and after six days of supplementation. The exercise regimen consisted of ten six-second bouts of high-intensity cycling at two exercise intensities, 130 revolutions per minute (rpm) or 140 rpm. There were no significant changes in any measured parameters in the placebo group. Those receiving the creatine supplementation had enhanced performance towards the end of each exercise bout at 140 rpm, as measured by smaller declines in work output with fatigue. The creatine group also demonstrated a mean body-weight gain of 2.4 pounds,

with no significant gain in the placebo group. It could be that the mechanisms responsible for the improved performance with creatine supplementation were due to both a higher initial muscle CP availability and an increased rate of CP resynthesis during recovery periods.

Greenhaff *et al.*, (1994), investigated the effect of supplementation of 20 g/day for 5-days on cycle performance in six healthy males (2 bouts \times 30 sec at 80 rpm and 4 minute rest interval) in a single-group ordered repeated measures design. The ingestion of creatine resulted in a 19% increase in $[Cr_{tot}]$ and a significant increase in the total work in the second cycle bout. They also noted a 50% reduction in ATP loss in the second exercise bout despite increased work performance, suggesting that a possible result of increased $[Cr_{tot}]$ is an attenuation of ATP degradation during high intensity physical activity.

Maximal isokinetic cycle performance (3 \times 30 sec at 80 rpm with 4min rest) was measured by Birch *et al.*, (1994) on 14 healthy males. The design was a double blind assignment to either placebo or creatine supplementation with testing before and after supplementation (see table 3). There were significant increases in peak power output (8% for bout 1), mean power output (6% for bout 1 and 2) and total work (6% for bouts 1 and 2) in the creatine group. When comparing the baseline group to the post-supplementation tests, there were no changes in bout 3 for the Cr group or any bout for the placebo group. Birch and colleagues (1994) thus concluded that cycling performance can be improved in the first two of three maximal 30 second bouts. Creatine ingestion also lead to a lower accumulation of plasma $[NH_3]$, thus suggesting an enhanced effect on muscle ADP rephosphorylation.

In Balsom *et al.*, (1995), seven physically active, male subjects were used for repeated bout tests (5 \times 6sec with 30 sec recovery periods) on a fixed intensity cycle ergometer. This was followed by a 10 second maximal bout to determine peak power output, before and after six days of creatine supplementation (25g/day). After supplementation the subjects were able to maintain a higher power output at the end of the 10 second bout.

The enhanced fatigue resistance after supplementation was associated with a greater [CP].

Dawson *et al.*, (1995), presented two studies in the same report. In the first they measured sprint performance (6 sec \times 6 bouts with a 24 sec recovery between bouts) on 22 subjects that were either taking creatine or a placebo. There were significantly greater increases in peak power output and total work in the creatine group. The creatine group also completed more work in sprint one (in isolation) compared to the placebo group, which is in contrast to the results for the 10 second performance test in the second study. In this study he randomly assigned 18 subjects in a double blind manner to either a placebo or creatine supplementation. He investigated the effects on single bout (10 sec) maximal ergo-cycle performance. They reported no differences between the groups following supplementation. This data can be difficult to explain, as the energy systems required for 6-second bouts are predominately ATP and CP, whereas glycolysis can already play a significant role in the 10 second maximum exercise and therefore reduce any effect of creatine supplementation.

Barnett *et al.*, (1996) also reported no effect of creatine supplementation on peak power output or mean power output during sprint cycle performance (7 bouts \times 10 sec) in 17 males randomized to either a placebo or creatine group.

In a single group design conducted on a cycle ergometer (2 bouts \times 30 sec at 80 rpm with a 4 min rest) in nine healthy males, Casey *et al.*, (1996), concluded that creatine supplementation resulted in a significant 19% mean increase in muscle [Cr_{tot}] as measured by a biopsy. Total work increased by approximately 4% in both bouts and the increases in both peak and total work were positively correlated with the increases in muscle [Cr_{tot}], specifically in Type II fibers. They also concluded that the improvements in work output were related to enhanced ATP resynthesis secondary to increased [CP] in Type II fibers.

Kirksey *et al.*, (1997), reported a significant 13% increase in the creatine group and a non-significant 5% increase in the placebo group in peak power in all five of the Wingate

trials (5×10 sec with 1 minute recovery). They concluded that creatine supplementation favourably increased power output.

In the study by Prevost *et al.*, (1997), 18 volunteers were divided into a creatine supplemented and placebo control group. Creatine supplementation consisted of 18.75 grams/day for 5 days followed by 2.25 grams/day for 6 days. Subjects were administered three different cycle ergometer intermittent interval training regimens, each with a work component at 150% of VO_{2max} . Creatine supplementation increased time to exhaustion by 61% for 30 second work/60 second rest and 62% for 20 second work/40 second rest for the second and third tests. A more than 100% increase in time for 10 second work/20 second rest (do as many as possible) first test was shown. The placebo group showed no change. They concluded that the ability to maintain high-intensity, intermittent exercise is enhanced by creatine supplementation.

Using a single-blind placebo control design and an exercise protocol of 5×15 -second maximal cycle ergometer bouts, Schneider *et al.*, (1997), concluded that creatine ingestion resulted in a significant 6.5% increase in total work compared to the placebo control group. Thus creatine supplementation may increase the rate of ATP resynthesis even in untrained subjects.

Of the 11 studies discussed previously on short term, high intensity (< 30 sec) work loads on cycle ergometers, 9 of the studies reported creatine supplementation having a successful ergogenic effect and 2 studies reported having no effect. This is sufficient evidence to show that creatine supplementation combined with such exercise protocols are successful.

5.6.2 High intensity, endurance exercise.

It has been hypothesized that increased levels of CP could reduce the reliance on anaerobic glycolysis as a replenishment source of ATP and possibly lead to decreased

formation of lactate and hydrogen ions and could therefore enhance performance in high intensity more prolonged exercise activities (60-150 sec)(Febbraio *et al.*, 1995).

Febbraio *et al.*, (1995), noted an increase in intra-muscular [Cr_{tot}] following creatine supplementation. Subjects were tested before and after supplementation (4×60 sec sprints followed by a 5th to exhaustion, all at 115-125% of VO_{2max}) and also re-tested after a 28-day washout period where the subjects consumed a placebo for the last 5-days. There were no differences in duration of the 5th exercise bout between baseline, post-supplementation and post-washout trials. They concluded that creatine supplementation has no ergogenic effect on exercise performance when the ATP-PCr energy system is not the principal energy source.

Jacobs *et al.*, (1997), reported an ergogenic effect in a moderately prolonged anaerobic exercise activity. Subjects were tested on a cycle ergometer, riding in one bout to exhaustion at 125% of VO_{2max} . They reported that the ride time to exhaustion was increased significantly following creatine ingestion from 131 to 143 seconds (8.5%), while the placebo group's time remained unchanged at 128 seconds. In physically active college students, Prevost *et al.*, (1997) reported a 24% increase (approximately 49 to 60 sec) in continuous cycle ergometer time to exhaustion at 150% of VO_{2max} following creatine supplementation (18.75 g/day for 5 days, then 2.25 g/day for 6 days). Both of these studies seem to contradict Febbraio *et al.*, (1995). However, Febbraio and colleagues test was done with the subjects already fatigued. A study design with two tests, one fatigued and one unfatigued would have been better in retrospect.

Schneider *et al.*, (1997), reported no improvement in cycle ergometer performance (5×60 sec all out effort) in nine untrained males following creatine supplementation. It can be that creatine supplementation is less likely to enhance performance of a maximal intensity more prolonged test (30-150 sec) due to energy system specificity. In tasks that rely primarily on fast glycolysis for ATP synthesis, the ergogenic effect of creatine could be limited. The difference between this study and the two that did not show an effect was that this study used an "all-out" protocol and measured work done, whereas the other 2

get an intensity and determined time. Thus it can still be debated whether such subtle differences in test protocols can alter the mechanism for fatigue and efficacy of creatine.

Barnett *et al.*, (1996), measured cycle ergometer VO_2 peak in 17 active volunteers before and after creatine supplementation. Creatine supplementation failed to increase the VO_2 peak.

In a study on cycling performance, Bellinger *et al.*, (2000) used 20 cyclists in either a creatine or placebo group in a randomized double blind manner. Creatine supplementation increased muscle [TCr], but did not increase the distance cycled in a one- hour distance trial. Godly and Yates, (1997), measured time to completion in a simulated 25km cycling race in which 16 well-trained male and female cyclists sprinted for 15 seconds every 4 km. There was no significant decrease in time to completion following creatine supplementation. They concluded that creatine supplementation has no effect on endurance activity, even when combined with short duration, high-intensity bouts in well-trained subjects. Thus it can be said that there is very little evidence that creatine supplementation will enhance performance in exercise activities dependent primarily on oxidative metabolism of endogenous carbohydrate and fat.

However, the controversy surrounding the shorter duration, high-intensity endurance tests makes this area still worthy of further investigation. In addition where is the cut-off between the two shorter endurance studies that were effective and the two long ones (25km and 1 hour) that were clearly ineffective? Also still a problem is that trained subjects were seldom used.

Table 5.6.1: The effect of creatine monohydrate supplementation on various Short term, high intensity (< 30 sec); cycle ergometer protocols on Exercise Performance.

Investigator, Year	N	Gender	Population	Design *	CM dose g/day	Days	Muscle Biopsy	Description	Ergogenic Effect?
Balsom <i>et al.</i> 1993a	16	M	Active/well trained	RDBPC	25	6	No	2 intermittent high-intensity (10×6)bouts before and after loading	Yes. Attenuated decline in power for bouts 7-10
Greenhaff <i>et al.</i> 1994	6	M	Healthy	SGRM	20	5	Yes	Isokinetic cycling (×2)	Yes. Increased total work in 2 nd test
Birch <i>et al.</i> 1994	14	M	Both highly and not highly trained	RDBPC	20	5	No	3×30 sec	Yes. Increase in peak power, mean power and work for bouts 1 and 2
Balsom <i>et al.</i> , 1995	7	M	Physically active	SGRM	20	6	Yes	5×6 sec; 1×10 sec	Yes. Increase in power during 10 sec trial
Dawson <i>et al.</i> 1995	18	M	Healthy active	RDBPC	20	5	No	1×10 sec	No.
Dawson <i>et al.</i> 1995	22	M	Healthy active	RDBPC	20	5	No	6×6 sec	Yes. Increase in peak power and Tot work
Barnett <i>et al</i> 1996	17	M	Recreationally active	RDBPC	20	4	No	7×10 sec sprint	No.

Casey <i>et al.</i> 1996	9	M	Healthy	SGRM	20	5	Yes	2×30 sec	Yes. 4% increase in peak power, 1% increase in Tot work
Kirksey <i>et al.</i> 1997	36	M/F	Track and field athletes	RDBPC	0.3g/kg/d	42	No	Wingate test ×5	Yes. 13% increase in mean peak power
Prevost <i>et al.</i> 1997	18	M/F	Active college students	RPC	18.75	5	No	Time to exhaustion at 150% VO ₂ max	Yes
Prevost <i>et al.</i> 1997 (2 nd study)					2.25	7		2 bouts each of 30swork/60s rest 20swork/40s rest 10swork/20s rest	Yes. 61% increase Yes. 62% increase Yes. 100% increase
Schneider <i>et al.</i> 1997	9	M	Untrained	RSBPC	25	7	No	5×15 sec	Yes. 6.5% increase in total work (kJ)

* RDBPC = randomized double blind placebo control; RPC = randomized placebo control; RSBPC = randomized single blind placebo control; SGRM = single group repeated measures

Table 5.6.2: The effect of creatine monohydrate supplementation on various; High intensity, more prolonged (>30 to <150 sec) cycle ergometer protocols on Exercise Performance.

Investigator, Year	N	Gender	Population	Design *	CM dose g/day	Days	Muscle Biopsy	Description	Ergogenic Effect?
Febbraio <i>et al.</i> 1995	6	M	Untrained	SGRM	20	5	Yes	4×60s; 115 –125 % VO ₂ max 5 th bout to exhaustion	No.
Jacobs <i>et al.</i> 1997	2 6	M/F	Physically active	RDBPC	20	5	No	125% VO ₂ max ride to exhaustion	Yes. 8.5% increase in time to exhaustion; 9% increase in max accumulated O ₂ debt
Prevost <i>et al.</i> 1997	1 8	M/F	Active college students	RPC	18.75	5	No	Time to exhaustion at 150% VO ₂ max	Yes. 24% increase
Schneider <i>et al.</i> .1997	9	M	Untrained	RSBPC	25	7	No	5×60 sec	No.

* RDBPC = randomized double blind placebo control; RPC = randomized placebo control; RSBPC = randomized single blind placebo control; SGRM = single group repeated measures

Table 5.6.3: The effect of creatine monohydrate supplementation on various aerobic (>150 sec) cycle ergometer protocols on Exercise Performance.

Investigator, Year	N	Gender	Population	Design *	CM dose g/day	Days	Muscle Biopsy	Description	Ergogenic Effect?
Barnett <i>et al.</i> 1996	17	M	Recreationally active	RSBPC	20	4	No	VO ₂ peak	No.
Bellinger <i>et al.</i> , 2000	13	M	Recreational Competitive	RDBPC	20	7	Yes	Cycle distance in 1 hour	No.
Godly and Yates 1997	16	M/F	Well trained cyclists	RDBPC	20	5	No	25km simulated road race with 6×15 sec sprints every 4km	No.

* RDBPC = randomized double blind placebo control; RPC = randomized placebo control; RSBPC = randomized single blind placebo control; SGRM = single group repeated measures

Chapter 6: Aims

The aims of this study were:

Study 1: To use the novel approach of using Peak Sustained Power Output (PSPO) as a predictor of cycling performance for variable fixed-workload testing and 5 km time trialing.

Study 2: Whether oral creatine supplementation would affect 5 km time trial performance and metabolism.

We hypothesized that creatine supplementation would affect time trial performance as well as the appearance of breakdown products of adenine nucleotides in the plasma.

Chapter 7: Methods and Materials

7.1 Subjects and ethical approval

Thirteen male subjects, between the ages of eighteen and thirty years of age, volunteered for the study. The cyclists were selected on their proven ability to complete a 105-kilometer cycle race in less than three hours and twenty minutes as well as being trained at the start of the study. All the subjects received a study information sheet outlining the study procedure (Appendix 1). The subjects were also required to complete an informed consent form (Appendix 2), which allowed them to withdraw at any stage of the study. The University of Stellenbosch ethical research committee for research with human subjects passed the study and approved the consent form (The ethical approval of the Committee of Ethical Evaluation of Research, Sub-Committee B, University of Stellenbosch, was given on 10 May 1999 for the application titled: "The effect of creatine-supplementation on stochastic exercise performance")

7.2 Experimental Procedure

7.2.1 Study 1: This study involved characterization of the metabolic response to a 5 km time trial as well as an investigation of physiological/biochemical and exercise predictors of performance. The test period was incorporated in the procedures for Study 2. See below.

7.2.2 Study 2: The study was a placebo controlled, single blind laboratory experiment to determine the effect of oral creatine supplementation on 5 km time trial performance and metabolism after stochastic testing. At no stage of the study were the subjects aware of whether they were receiving active agent or placebo and were only made aware of contents at the end of the sample analyses.

The test period was 4 weeks long. The first week consisted of the initial Peak Sustained Power Output (PSPO) test, conducted on an electromagnetically braked cycle ergometer (Technogym®, Bikerace HC 600, Gambettola, Italy) in order to assess the fitness and cycling power of the cyclists. Two days later the subjects returned to the laboratory with their own bicycles to conduct the first of two baseline stochastic tests, each followed directly by a high intensity 5 km time trial in the quickest possible time in each case (ST1 and ST2). The stochastic tests were conducted on an electromagnetically braked cycle ergometer (Technogym®, Bikerace HC 600, Gambettola, Italy), followed directly by a 5 km time trial on a cycle ergometer (Technogym®, Spintrainer, Gambettola, Italy).

In the second week the subjects completed two 5 km high intensity time trials (TT1 and TT2) on the cycle ergometer (Technogym®, Spintrainer, Gambettola, Italy). They were to complete the test as quickly as possible and the tests were conducted two days apart. During TT2 blood samples were taken for plasma lactate and plasma hypoxanthine and urate. A muscle biopsy was taken after TT2 for intramuscular total creatine levels, histological ATPase fiber typing and SDS gel electrophoresis for MHC separation.

The 13 subjects were then given sachets containing an unknown substance. Seven subjects received the active agent, an oral creatine supplement, 5 grams (Phosphagen, Experimental and Applied Sciences (EAS), ABG, Cape Town) mixed with 20 grams of glucose polymer (Refuel cc., Observatory, Cape Town) and 6 subjects received sachets containing 25 grams of a placebo (glucose polymer). The powders were the same texture, colour and mass. The contents of the sachets were mixed with water and 4 sachets were consumed 4 times a day. The subjects were then to load for seven days (third week) while continuing with their regular training program.

In the fourth and final week the subjects returned and conducted another stochastic test with a 5 km time trial directly afterwards (ST3). Two days later the subjects returned for a single 5 km time trial (TT3) where blood samples were once again taken for the

analysis of plasma lactate and plasma hypoxanthine and urate. Approximately 1-2 hours later a second muscle biopsy was taken for intramuscular TCr and for muscle fiber typing.

7.3 The Peak Sustained Power Output (PSPO) Test

The test was conducted on an electromagnetically braked cycle ergometer (Technogym®, Bikerace HC 600, Gambettola, Italy) that had been modified to have racing handlebars (Acor race-bars), racing seat (Giant, gel-system) and cleated pedals (Look, clipless pedal system). The cycle ergometers' seat height and handle bar height was adjusted for each subject and recorded for further reference. The subjects' heart rate was monitored using a heart rate recording/transmitter belt (Polar®, Finland) that is compatible with the Technogym® software on the ergometer's computer.

The test commenced with a warm up of up to 10 minutes. The initial exercise intensity was calculated at 3.33 W per kilogram body mass and was maintained for 150 sec according to the protocol of Hawley and Noakes, (1992), thereafter the workload was increased with 50 W for another 150 sec and thereafter by 20 W until exhaustion. The cyclists remained seated for the entire duration of the test and maintained a cadence of 70 revolutions per minute (rpm) or more. If this could not be maintained the test was ended. If the last workload could not be completed the peak sustained power output was calculated according to the formula:

$$\text{PSPO} = \text{Workload (previous)} + [(\text{time completed in final workload} / 150) \times 20 \text{ W}]$$

The following equation from Hawley and Noakes, (1992). could also be used to calculate an 'estimated' VO_2 peak. According to this protocol the correlation reported was very close between peak workload and $\text{VO}_{2\text{max}}$ ($r = 0.97$, $P < 0.0001$).

$$\text{VO}_{2\text{peak}} (\text{calculated}) = 0.01141 \times \text{PSPO (W)} + 0.435$$

(Multiply 1000 to obtain the answer in ml/min/kg).

The subjects' power to weight ratio was also calculated:

Power: weight = PSPO/ body mass = W/kg

This ratio gives an indication of the athlete's training status and racing potential. It was also used as a variable for correlation with their time trial performance time and other variables measured during the project.

7.4 The Variable fixed-workload protocol and 5 kilometer Time Trials

Prof. K.H. Myburgh and Mr. C. de Klerk designed this variable fixed-workload protocol . The test was designed and structured to include some submaximal steady state workloads (Appendix 3). These were interspersed by shorter time periods at either low intensity or supra-maximal intensity workloads and followed directly by a 5 km time trial as the final workload. The stochastic test was conducted on an electromagnetically braked cycle ergometer (Technogym®, Bikerace HC 600, Gambettola, Italy) and the 5-kilometer time trial on their own bicycles linked to a cycle ergometer (Technogym®, Spintrainer, Gambettola, Italy). The exercise intensity was expressed as a percentage of the peak power output. The subjects' heart rates were monitored and recorded after each workload using a Polar® heart rate electrode belt that is compatible with the Technogym® software. Each subject conducted two such tests before their loading week (ST1 and ST2) and one more after the loading week (ST3). Performance was taken as the duration of the 5 km time trial, since all other workloads were for set durations and the same for each subject in each test.

The subjects were also required to complete 5 km time trials without a pre- variable fixed-workload test. These were conducted on their own bicycles, that were attached to a cycle ergometer (Technogym®, Spintrainer, Gambettola, Italy). Two tests were conducted before the loading week (TT1 and TT2). TT1 was used as familiarization time trial and for repeatability of the tests. During and after TT2, blood samples and muscle

biopsies were taken. A third and final individual time trial (TT3) was conducted after the loading week where once again blood samples were taken as well as a muscle biopsy.

7.5 Sample Handling and Analyses

7.5.1 Blood.

Blood samples were taken at specific time points before, during and after TT2 and TT3. Blood was drawn using an in-dwelling cannula (Jelco™, 22G, Johnson and Johnson medicine, RSA) and stopcock (RE/NM/stopcock, GRS medical, RSA) inserted into a forearm vein. The cannula was flushed with sterile saline (0.9% sodium chloride, Adcock Ingram, Johannesburg, RSA) containing 1ml of heparin Novo (Novo Nordisk (Pty) Ltd., Johannesburg, RSA) per 100ml saline.

Blood samples for plasma lactate were taken at rest, 3 minutes into the time trial, at the end of the time trial and at 2, 4, 7, 10 and 20 minutes of recovery. Blood samples for plasma hypoxanthine and urate were drawn at rest, at the end of the time trial and at 4, 10 and 20 minutes of recovery. Prior to taking each sample, approximately 1 ml of blood was drawn and discarded, due to the “dead space” from the saline flushing. Following this approximately 4 ml of blood was drawn on each occasion with a syringe. The blood was then aliquotted in 5 ml evacuated tubes (Vacutainer, Becton Dickson, Plymouth, UK) mixed well with the contents of the tubes and kept on ice. Two types of evacuated tubes were used, a Grey-top tube (containing sodium fluoride and potassium oxalate) for lactate analysis and a green-top tube (containing lithium and heparin) for the analysis of hypoxanthine and urate. The blood was then centrifuged (Sarstedt LC 1-K centrifuge, Optolabor, Randburg, RSA) at 3000 rpm's for 10 minutes at 3°C. After separation, 1ml of plasma was removed using disposable Pasteur pipettes (Volac, John Poutten Ltd., Essex, England) and placed in marked 1.5 ml Eppendorf storage vials and stored at –20°C for later determination of lactate, hypoxanthine and urate concentrations.

Lactate was analyzed using a commercially available kit (Lactate PAP, BioMerieux, Separation Scientific, Honeydew, RSA) and spectrophotometer (Cary 50 conc, UV

visible spectrophotometer, Varian Australia (Pty) Ltd., Mulgrave, Australia). Pippetting was done using digital micro-pipettes (Autoclavable Nichipet, Model 5000DG, Nichiryo, Tokyo, Japan). Lactate concentrations were calculated using Microsoft Excel™ spreadsheets. There was less than a 3% error in repeatability in 90 samples done in duplicate. A further 14 samples were between 3-5% in error. These were repeated in duplicate and in all cases the error was then less than 3%. ($\% \text{ error} = \frac{\text{ABS1} - \text{ABS2}}{(\text{ABS1} + \text{ABS2})/2} * 100$).

The determination of hypoxanthine and urate was done using the High-performance liquid chromatography (HPLC) analyses. Plasma hypoxanthine and urate were analyzed using a Gilson Instruments HPLC (Gilson Inc., Middleton, U.S.A). It consisted of a Gilson 305 pump, a Rheodyne 7125 injection valve (Rheodyne, California, U.S.A) and Gilson UV/VIS detector (119UV). The column used was a Phenomenex Ultracarb 5 ODS (two linked 150 x 4.6 mm columns). This analysis method utilized a mobile phase of phosphate buffer. The mobile phase buffer was made up of 90 mM potassium dihydrogen phosphate buffer (ACS reagent grade, ICN Biomedical Inc., Ohio, USA), 5% HPLC-grade acetonitrile (BDH, Hypersolv, England) and 2.3 mM tetra-butyl-ammonium hydrogen-sulphate (Merck, Schuchardt, Germany). The buffer was then titrated to pH 6.5 using 40% KOH and made up to volume using distilled 18 MOhm water. Following this the buffer was filtered and degassed. The flow rate was set at 0.9 ml/min and the total run time was 8 minutes. Integration of the peaks was only begun after 4 minutes of the run. Stock standards were made up to volume with mobile phase buffer and frozen. These were defrosted at the start of every analysis day. The plasma samples were extracted and this method involved adding 100 µl of plasma to 100 µl of ice-cold 0.6M perchloric acid (Merck, Germany). The sample was then centrifuged in a micro-centrifuge (Beckman Microfuge® Lite, Beckman, Germany) for 4 minutes at 11500 rpm. Following this 150 µl of supernatant was removed and neutralized with 9 µl of 2.5 M K₂CO₃ anhydrous (BDH Laboratory supplies, Poole, England). This sample was then placed on ice for 10 minutes and again centrifuged for 4 minutes at 11500 rpm. After centrifugation 100 µl of supernatant was drawn off and made up to 500 µl with 400 µl mobile phase buffer. The

mobile phase buffer was made up to be the same as the buffer discussed previously of 90 mM potassium dihydrogen phosphate buffer (ACS reagent grade, ICN Biomedical Inc., Ohio, USA), 3.5% HPLC-grade acetonitrile (BDH, Hypersolv, England) and 2.3 mM tetra-butyl-ammonium hydrogen-sulphate (Merck, Schuchardt, Germany). The buffer was again titrated to pH 6.5 using 40% KOH and made up to volume using distilled 18 Mohm water. The sample was then injected onto the column. The injection volume was 20 μ l of sample. Both hypoxanthine and urate were detected at 249 nm and analysis was performed according to area. This method provided good resolution peaks in both standard and sample runs.

7.5.2 Muscle.

Muscle biopsies were taken on two occasions once after TT2 and once after TT3. The samples were removed from the vastus lateralis muscle using a stainless steel Trepine biopsy needle (6 mm). The muscle biopsies were performed under sterile conditions and performed by a medical doctor with experience in this procedure. Prior to incision the skin was prepared and cleaned with a disinfectant (Betadine® antiseptic solution, Adcock Ingram Pharmaceuticals, Johannesburg, RSA) for administration of local anaesthetic. Four milliliters of local anaesthetic (Peterkaien, 2% m/v injection, Intramed (Pty) Ltd., Port Elizabeth, RSA) was injected below the skin to the level of the muscle fascia and sufficient time was allowed for the anaesthetic to take effect. A scalpel incision was then made through the skin and fascia to the level of the muscle. The muscle biopsy needle was then inserted and two muscle samples taken immediately, the second after turning the needle $\pm 90^\circ$. The first muscle sample was placed in liquid nitrogen within 10 seconds of the biopsy needle being removed from the vastus lateralis and the second sample was mounted on cork and Tissue Tek, a mounting compound, was applied to the sample where it was then placed in semi-frozen isopentane for muscle histochemistry.

Pressure was then applied to the wound until the bleeding stopped. The wound was again disinfected (Betadine® antiseptic solution, Adcock Ingram Pharmaceuticals, Johannesburg, RSA) and closed using Steristrip™ skin closures (3M, reinforced skin closures, 6mm × 75mm, Borken, Germany). This was then covered with Tegaderm™ (3M, transparent dressing, 6cm × 7cm, Ontario, Canada). The leg was then bandaged (Premier, conforming bandage, 75mm, manufactured by Megrotex, RSA) and the subject was then given Paracetamol (500mg) tablets for pain relief as the anaesthetic subsided, to be taken 1 or 2 tablets every four hours as needed.

The first muscle sample that was frozen in the liquid nitrogen was then placed in Cryovials™ (Greiner labortechnik), weighed and stored at -80°C until analysis for total creatine (TCr) and muscle nucleotide content, which was conducted using the HPLC method. Before the muscle could be used for HPLC analysis the samples had to be freeze-dried in a vacuum (Christ LDC-1 freeze-dryer with sliding vane rotary vacuum pump, Vacubrand, Wertheim, Germany) in little glass containers which could be stored until analysis at either room temperature or -20°C, without breaking the vacuum seal until just prior to weighing the sample. At this time, the container needed to be equilibrated to room temperature.

The HPLC method is based on a combination of that described by Sellevold *et al.*, (1986) and Ally and Park (1992). Certain modifications were made to provide better resolution of peaks. A Gilson Instruments HPLC (Gilson Inc., Middleton, U.S.A) was used. It consisted of a Gilson 305 pump, a Rheodyne 7125 injection valve (Rheodyne, California, U.S.A) and Gilson UV/VIS detector (119UV). The column used was a Phenomenex Ultracarb 5 ODS (two linked 150 x 4.6 mm columns). The flow rate was set at 0.9 ml/min for the full run time of 30 minutes. A gradient of 20% acetonitrile buffer B was also run during the 30 minutes. This buffer was injected at 6-minutes and the percentage of the two buffers was at 50% each at the 13th minute and continued as such until the 23rd minute and then buffer B slowly decreased to 0% by the 26th minute and the run then continued with buffer A till minute 30. The injection loop consisted of 20 µl of

sample. This method used consisted of a mobile phase of phosphate buffer, the same used for the plasma analysis, made up using 90 mM potassium dihydrogen phosphate buffer (ACS reagent grade, ICN Biomedical Inc., Ohio, USA). The buffer was then filtered and degassed. Standards were made up using mobile phase buffer and were divided into aliquots and frozen at -18°C . Fresh standards were defrosted every morning and mixed together to form a combination of all the compounds expected in a muscle sample run. Thus a high and a low standard mix were made up for injection onto the column. Standard runs were repeated at regular intervals during an analysis day.

The creatine values had to be corrected to an equal nucleotide (ADP and ATP) value for each subject's pre- and post-loading biopsy before the comparisons between the placebo and creatine groups could be made (see chapter 8 for discussion).

The second muscle sample that was frozen in isopentane was used for muscle histochemistry. It is a delicate freezing procedure (Appendix 4) that had to be conducted correctly for ideal muscle sections. The mounted sample was then cut into $10\ \mu\text{m}$ sections in a cryostat (Cryostat) at -18°C . Sections were mounted on cover glasses or glass slides. The sections were then allowed to air dry for at least 30-minutes before staining or were alternatively stored at -80°C to be stained the next day.

Myosin ATPase staining was conducted using the method by Brooke and Kaiser, (1970a). It consisted of 3 buffers being made up: A veronal buffer with pH 9.4 and a 0.2 M acetate buffer with pH 4.3 and pH 4.6 respectively. A substrate incubation solution with pH 9.4 was also made up (Appendix 5). With this method the result was that 3 fibre types, I, IIA and IIB (also called IIX), were discriminated between and thus the fibers could be typed.

The muscle samples were also analyzed using the SDS-polyacrylamide gels, used for myosin heavy chain (MHC) separation. In this method the muscle samples that were used were cut from a Tissue-Tek embedded biopsy (15 sections of $15\ \mu\text{m}$ each) and placed in

a denaturation buffer (Appendix 6). Two SDS-gels were used to run the samples, a separating gel and a loading gel (Appendix 6). In both the separating and loading gels polymerization was initiated by ammonium per-sulphate/TEMED (Fry *et al.*, 1994). The gels were run for 48 hours at 70 volts. Subsequently the gels were stained with Coomassie R250 and the relative amount of MHC isoforms were quantified using a densitometric system (Cream 1-D, Kem-en-Tec Aps, Copenhagen, Denmark).

7.6 Statistical Analysis

Statistical analysis was performed on NCSS 2000 statistical system (Number Cruncher Statistical Systems, Kaysville, Utah, USA). Repeated measures ANOVA tests were conducted for the analysis of the blood metabolites. The data in the tables were presented as mean values and standard deviations (SD) of the mean. Students' t-test was used where appropriate. The accepted level of significance was $P \leq 0.05$. Results were also recorded as mean \pm 1 standard deviations (SD).

CHAPTER 8: RESULTS

8.1 Subjects: Thirteen male subjects volunteered for this study. All subjects were trained cyclists. The study fell within the cycling season (summer). All subjects had completed a 105 km race within the previous racing competitive season and were training for the same race at the time of participation in the study. Subject characteristics are presented in Table 1.

TABLE 1: Subject Characteristics.

Subject	Age	Mass (kg)	Height (cm)	105km time (min)	PPO	PSPO/kg
1	18	72	185	169	324	4.5
2	25	74	176	167	368	5.0
3	18	68	175	196	336	4.9
4	26	73	184	182	380	5.2
5	18	56	160	181	273	4.9
6	22	73	187	184	383	5.2
7	22	88	197	197	395	4.5
8	20	67	187	200	352	5.2
9	20	68	180	179	336	4.9
10	18	67	181	172	340	5.1
11	20	67	185	181	345	5.2
12	19	64	182	179	323	5.1
13	25	80	180	199	360	4.5
Mean	20.8	70.5	181.5	183.5	347.3	4.9
SD	2.9	7.8	8.5	11.3	31.9	0.3

PSPO = Peak Sustained Power Output

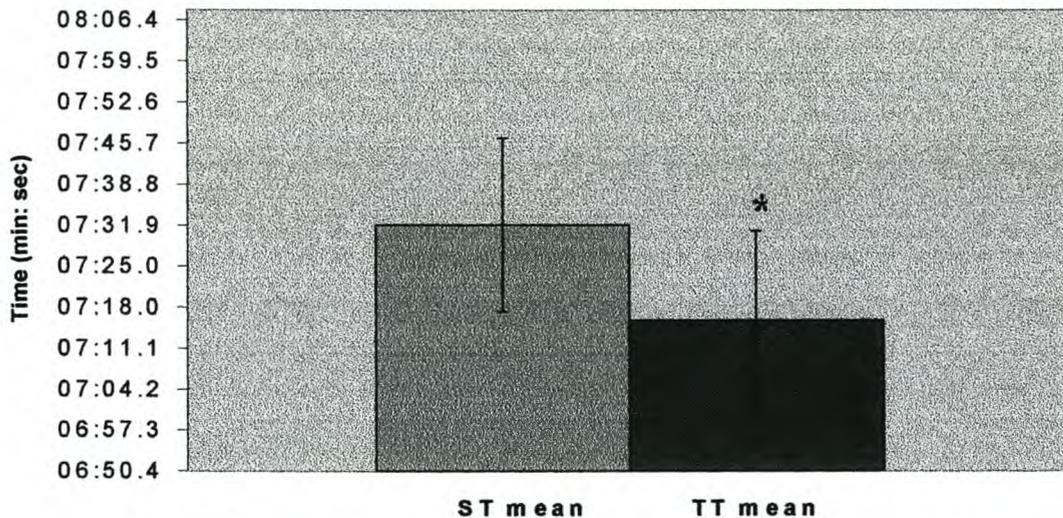
SD = Standard Deviation

8.2 STUDY 1

8.2.1 Performance data:

The major laboratory performance trial was the 5 km time trial. The repeatability of the 5 km TT was good: the percentage error between TT1 and TT2 for individual subjects ranged from -0.95% to 1.8%. The coefficient of variation was: 0.6% for all subjects. Therefore, we used the average of TT1 and TT2 as the major performance measure, hereafter called TT mean.

The 5 km TT was also performed twice under fatigued conditions, after 32 minutes of stochastic exercise. The repeatability was also good: the percentage error between ST1 and ST2 for individual subjects ranged from -2.19% to 1.12%. The coefficient of variation was 0.7%. Therefore, we used the average of ST1 and ST2 as the major measure for performance under fatigued conditions. There was a significant difference between 5 km TT performed fresh (TT mean) and 5 km TT performed fatigued (ST mean) ($P=0.0001$). For mean values see Fig. 1. There was definite fatigue with a decrement in time ranging between 1.0 sec to 38.0 sec for individual subjects with a mean of 18.2 ± 11.9 sec (SD).

FIGURE 1: The 5-km time trial performances.

* $P = 0.0001$ (Table of means \pm SD in Appendix 8)

Abbreviations:

ST mean = the mean value of two 5 km time trials (ST1 and ST2) performed under fatigued conditions after 32 minutes of stochastic testing.

TT mean = the mean value of two 5 km time trials (TT1 and TT2).

8.2.2 Relationship between different measures of exercise performance:

PSPO is usually used as the “gold standard” for determining endurance performance in the laboratory. We determined the relationship between two different high intensity endurance performance tests: PSPO and 5 km TT (TT mean). The correlation was: $r = -0.79$ $P < 0.01$. The correlation between PSPO and the 5 km TT performed in the fatigued condition (ST mean) was slightly worse: $r = -0.60$ $P < 0.05$.

We also related power to weight ratio (PSPO/kg) to time trial performance, but there were no significant correlations : PSPO/kg vs. 5 km TT was $r = -0.26$ and PSPO/kg vs. 5 km TT in the fatigued state: $r = -0.47$.

Performance in the 105 km race was also not related to any of the laboratory performance measures.

TABLE 2: Non-significant correlations between 105 km race time and different laboratory measures of exercise performance.

	105 km
PSPO	$r = 0.25$
TT mean	$r = -0.21$
ST mean	$r = -0.24$
PPO/kg	$r = 0.08$

The relationship between the physical variables (age and mass) and performance variables (105 km time, PSPO, TT mean and ST mean) were correlated. A good relationship was found between age and PSPO ($r = 0.73$; $P < 0.05$). Age also showed a good negative relationship with TT mean ($r = -0.71$; $P < 0.05$). A very good relationship was found between body mass and PSPO ($r = 0.85$; $P < 0.01$) but not between body mass and TT mean. No significant relationships were found between the physical variables and 105 km time and STmean

8.2.3 Metabolic variables:

Plasma lactate concentrations were determined at various time points during TT2 (Fig. 2). Plasma lactate concentrations were significantly different from rest at all other time points ($P < 0.0001$). The other time points were at 3-minutes into the time trial and at recovery from time zero to 20 minutes (see Fig. 2). Hypoxanthine ($P < 0.0001$) and urate ($P = 0.05$) concentrations were also significantly different from rest at all other time points. The time points recorded were at rest and during recovery at time zero, 4, 10 and 20 minutes. (see Fig. 3 and Fig. 4). Thus the repeated measures ANOVA reached significance and that post hoc testing showed the resting value to be significantly different to all other values. Using the data provided in the appendix, there were differences between many of the other time periods.

FIGURE 2: The concentrations of plasma lactate before, during and after TT2. (Table of means \pm SD in Appendix 9). * ANOVA, $P < 0.0001$

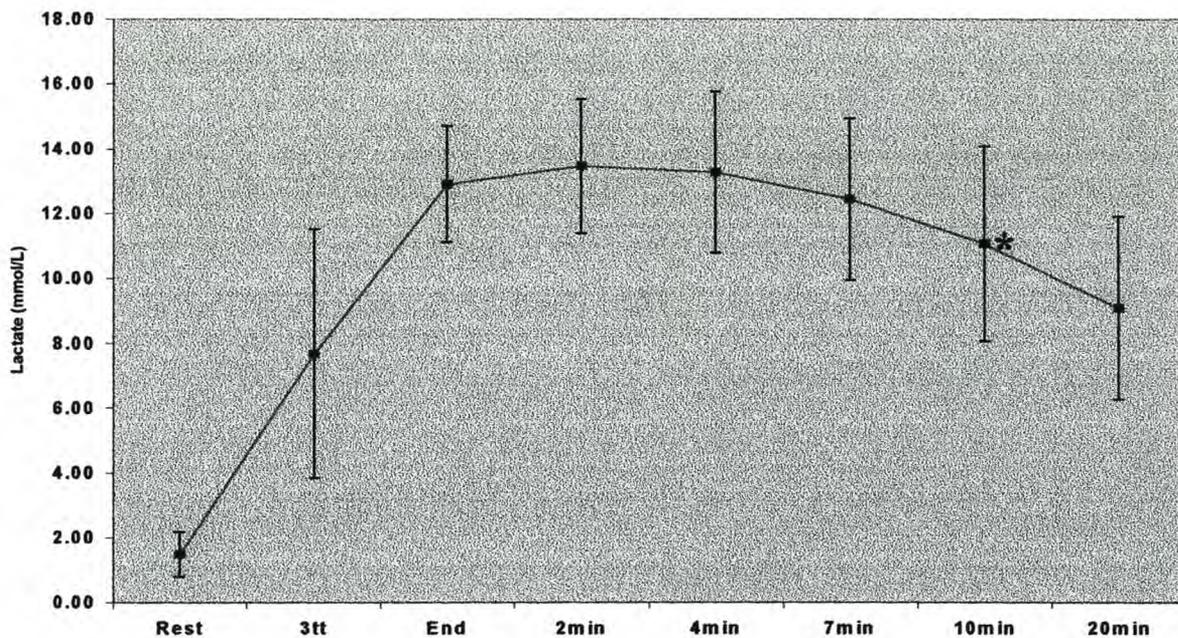


FIGURE 3: Concentration of plasma hypoxanthine before and after TT2. (Table of means \pm SD in Appendix 9). * ANOVA, $P < 0.0001$

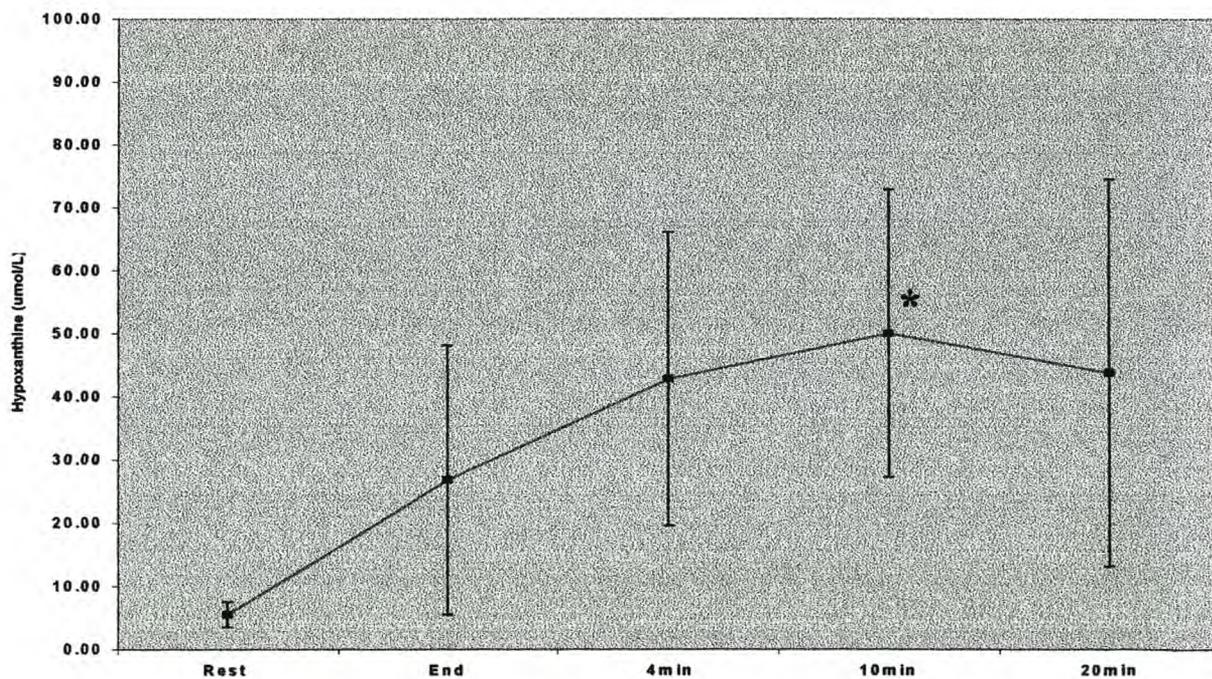
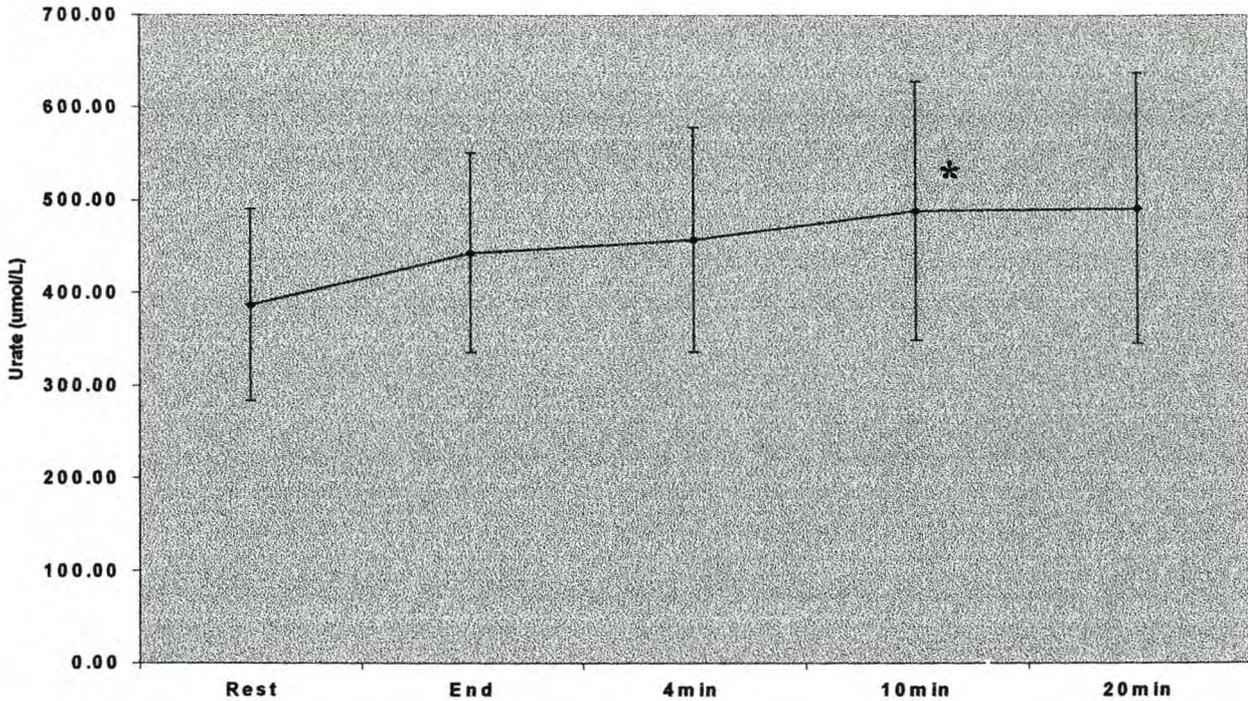


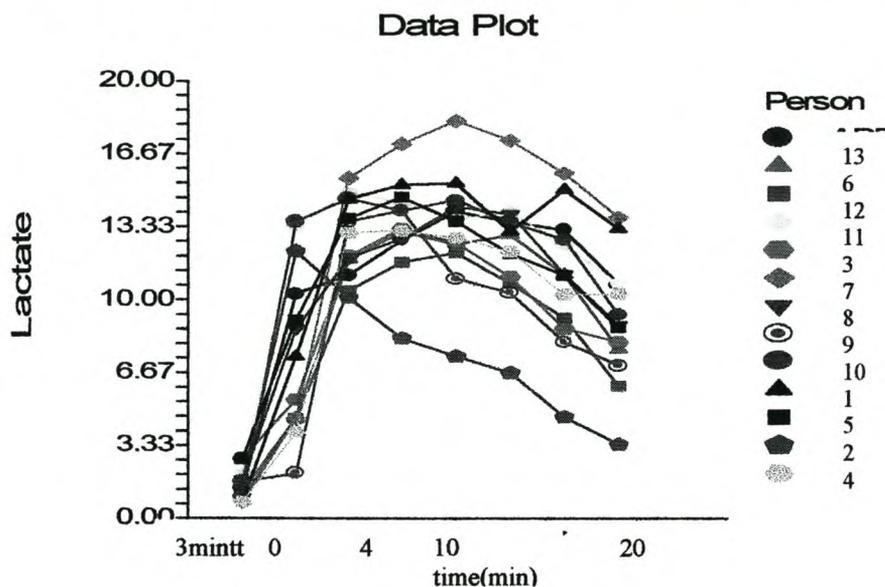
FIGURE 4: The concentrations of plasma urate before and after TT2. (Table of means \pm SD in Appendix 9)

*** ANOVA, P=0.05**



8.2.4 Relationships between exercise performance (TT2) and metabolic variables.

The relationship between recovery time and metabolite concentrations was notable for plasma lactate. The plasma lactate concentrations were at their highest at times 0, 2 and 4 minutes, but there was hardly any recovery in the concentration of plasma lactate for 20 minutes following this high intensity endurance exercise bout. The area under the curves for the lactate concentrations for all the subjects, (Fig. 5), were calculated and the results were correlated with TT2. The result was a weak non-significant correlation of $r = 0.28$.

FIGURE 5: Individual curves for the subjects (n=13) for plasma lactate concentration.

Plasma hypoxanthine levels were also affected by the intensity of the 5 km time trial (TT2). Hypoxanthine concentrations reached their peak at 10 min post-exercise. The area under the curve for hypoxanthine was calculated for all the subjects (n=13) and correlated with TT2 and a non-significant correlation of $r = -0.16$ resulted.

The plasma urate concentrations also increased due to the intensity of TT2. The concentrations did not increase considerably in comparison to the resting values but the concentrations remained at a high level and plateaued even after 20 minutes of recovery. The correlation of the area under the curve for urate and TT2 performance time resulted in a non-significant value of $r = -0.33$.

8.2.5 Muscle physiology variables:

We determined myosin heavy chain composition in homogenates from a portion of the muscle biopsies frozen in Tissue-Tek. Percentage MHC I was 58.3 ± 10.6 (SD) and

percentage MHC IIa was 40.8 ± 11.0 (SD). Only one subject had any type IIx fibres (11.7%).

Histological fibre typing was done for one subject as a case study. The results for the ATPase staining method were fiber type I: 42%, type IIA: 57% and type IIB: 1%. The same subject's %MHC isoform distribution was %MHC I = 37.6 and %MHC IIa was 62.4.

There were no significant relationships between exercise performance variables and muscle physiology variables.

TABLE 3: Non-significant correlations between %MHC composition and exercise performance variables.

	MHC I	MHC IIa
PSPO	$r= 0.06$	$r= 0.15$
PSPO/kg	$r= 0.04$	$r= -0.03$
105-km	$r= -0.24$	$r= 0.26$
ST mean	$r= -0.18$	$r= -0.01$
TT mean	$r= -0.28$	$r= 0.18$

There were significant correlation between plasma lactate concentration at time 0 in recovery (i.e. at completion of TT2) and % MHC I ($r=0.59$, $P<0.05$) and % MHC IIa ($r= -0.61$, $P<0.05$). The areas under the curve for lactate resulted in non-significant correlations of $r= 0.16$ and $r= -0.10$ for %MHC I and % MHC IIa respectively.

There were significant correlations between plasma hypoxanthine concentration and %MHC I and %MHC IIa at time 0 and time 10 min during recovery. Area under the

curve for hypoxanthine showed significant relationships of $r = -0.53$ ($P = 0.05$) and $r = 0.56$ ($P < 0.05$) respectively for %MHC I and %MHC IIa.

Plasma urate concentrations at the same time points also showed no significant correlations with %MHC I and %MHC IIa. The correlation between %MHC I and the areas under the curve for the subjects had a non significant correlation $r = 0.04$

8.3 STUDY 2:

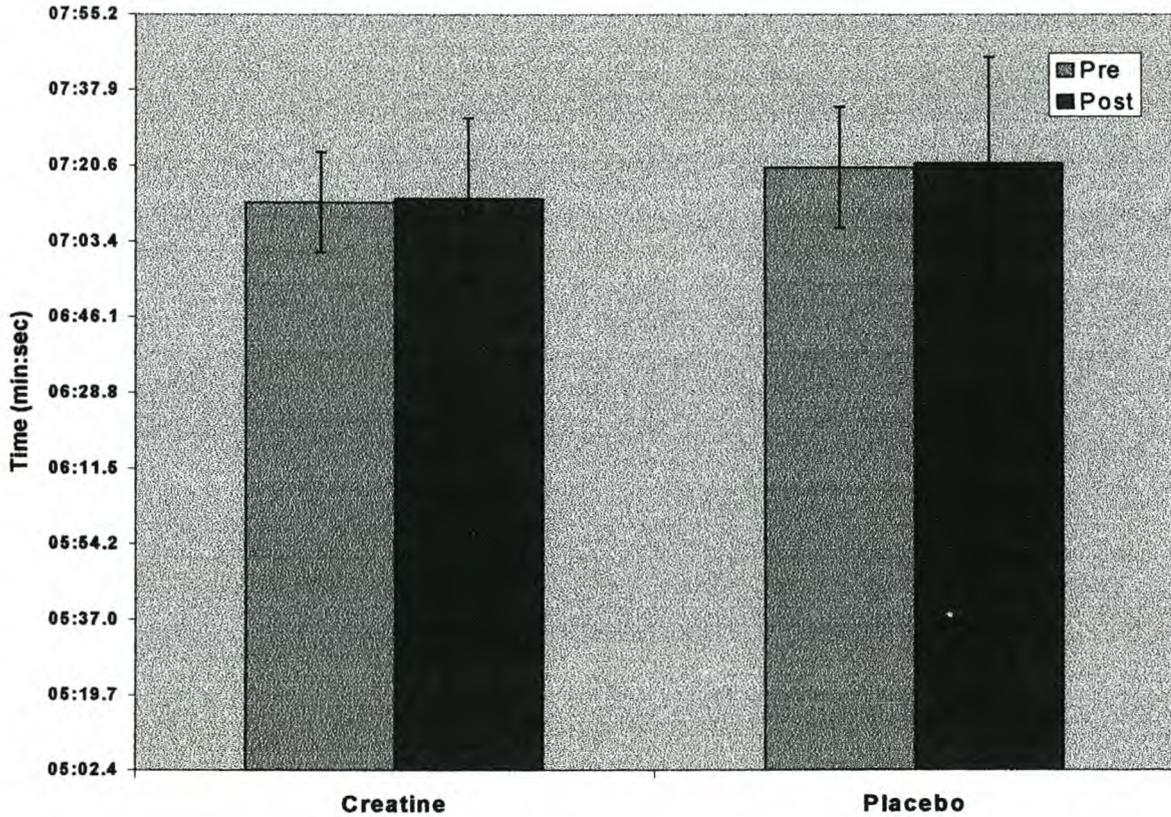
The same 13 male subjects participated in a nutritional intervention trial to determine whether oral creatine supplementation would influence 5-km time trial performance in a fresh as well as in a fatigued state. After randomisation it transpired that 7 subjects were in the creatine group and 6 subjects in the placebo group. The group characteristics are in the underlying table and presented as means \pm SD. There were no statistically significant differences between the two groups for any of these variables.

TABLE 4: Subject characteristics for the creatine (n=7) and placebo (n=6) groups. (Statistical analysis by unpaired Students t-test: not significant).

	Age (yrs)	Mass (kg)	Height (cm)	105km time (min)	PSPO (W)	PSPO/kg
Placebo	20.33 ± 2.73	69.83 ± 5.78	182.00 ± 4.56	188.33 ± 11.67	349.00 ± 21.03	5.02 ± 0.28
Creatine	21.29 ± 3.20	71.14 ± 9.60	181.00 ± 11.28	179.43 ± 9.86	345.86 ± 40.73	4.88 ± 0.28

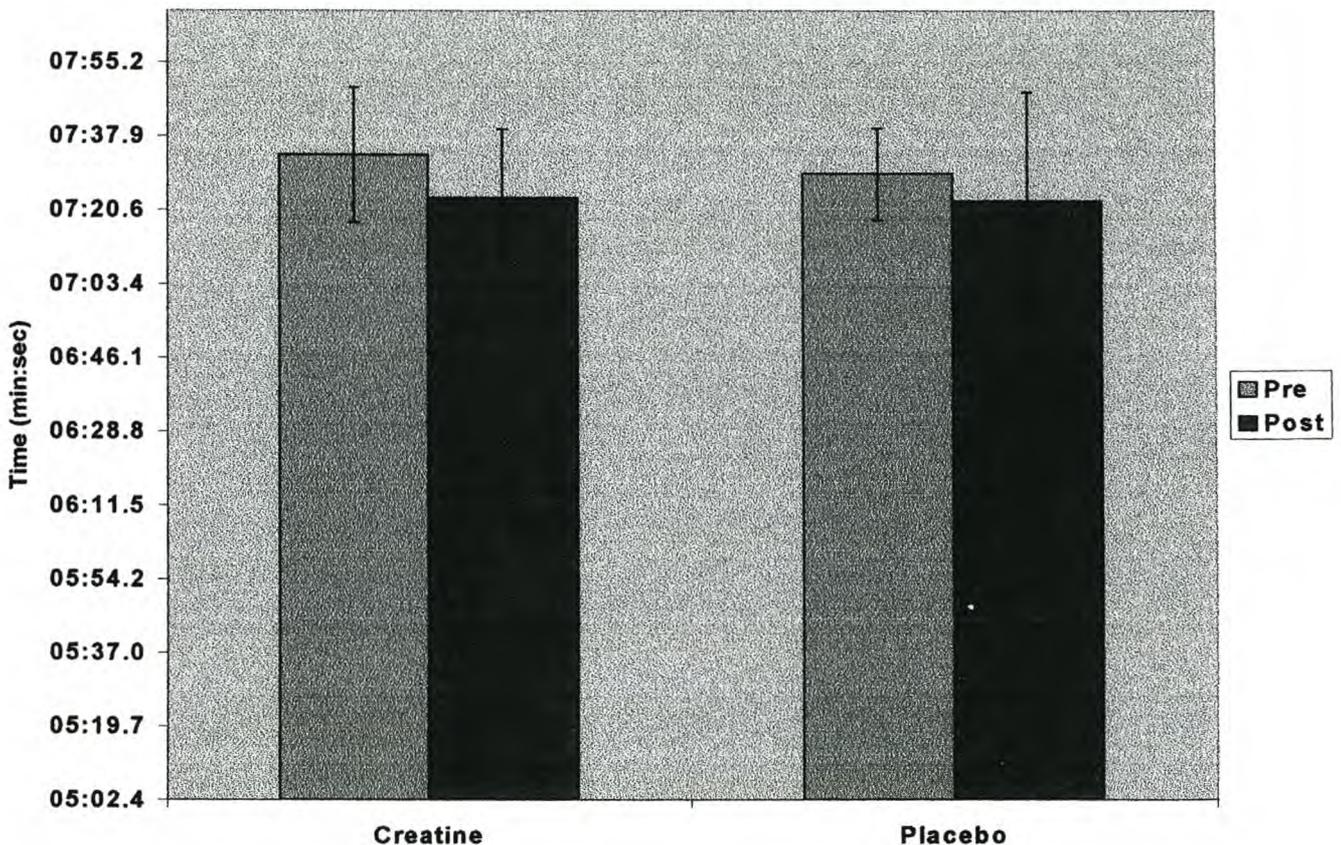
Performance times were compared for the 5 km time trials: TT mean and TT3. The times recorded after the loading week were slightly slower for both the placebo and creatine groups, but not significantly so. The 5 km performance times recorded by the creatine group were, pre-loading time 7 min 12 sec \pm 11.4 sec (SD) and post-loading 7 min 13 sec \pm 13.8 sec and the placebo control group recorded times for pre-placebo 7 min 20 sec \pm 18.3 sec and post-placebo 7 min 21 sec \pm 24.3 sec (see Fig. 6). When looking at individual results, 2 subjects in the placebo group were slower for TT3 than TTmean and 4 subjects in the creatine group were for TT3 than TT mean.

FIGURE 6: The comparison of 5 km time trial performance for the creatine (n=7) and placebo (n=6) groups before and after 7 days of creatine loading or placebo. (Table of means \pm SD in Appendix 8)



A 5 km time trial in the fatigued state was also conducted (ST3) after the loading phase. This performance time was compared to ST mean. The creatine group showed a non-significant improvement in performance time after the 7-day loading period (pre-load 7 min 34 sec \pm 14.5 sec and post-load 7 min 23 sec \pm 9.8 sec). The placebo group also showed a non-significant improvement in performance time (pre-placebo 7 min 29 sec \pm 16.0 sec and the post-placebo 7 min 23 sec \pm 25.4 sec) (see Fig. 7) (Table of means \pm SD in Appendix 8). When looking at individual results, two subjects in each group had slower ST3 than ST mean, whereas the other subjects were faster.

FIGURE 7: The comparison between 5 km time trial performance times in the fatigued state between the placebo (n=6) and creatine (n=7) groups before and after 7 days of creatine loading and placebo.



8.3.1 Nutritional intervention and metabolic variables.

Plasma hypoxanthine and urate samples were taken at the post-loading 5 km time trial (TT3). The levels of these plasma metabolites were compared to the concentrations of plasma hypoxanthine and urate that were sampled at TT2 and between the creatine and placebo groups (see Fig. 8 and Fig. 9). The plasma hypoxanthine levels were significantly different from rest at the recovery time points zero, 4, 10 and 20 minutes in both the creatine and placebo groups ($P < 0.0001$). The creatine and placebo groups were not different from each other for either the pre- or post tests or the response over time.

Plasma urate concentration changed over time ($P < 0.005$) and the time effect was not different for pre- and post tests. There was a significant group effect with the creatine group showing lower urate concentrations in the post test that was not apparent for the placebo group who tended to have higher values for the post test.

Figure 8: The comparison of plasma hypoxanthine concentrations pre- and post the 7 day loading period in the placebo ($n=6$) and creatine ($n=7$) groups. (Table of means \pm SD in Appendix 9). * $P < 0.0001$ (significant time effect)

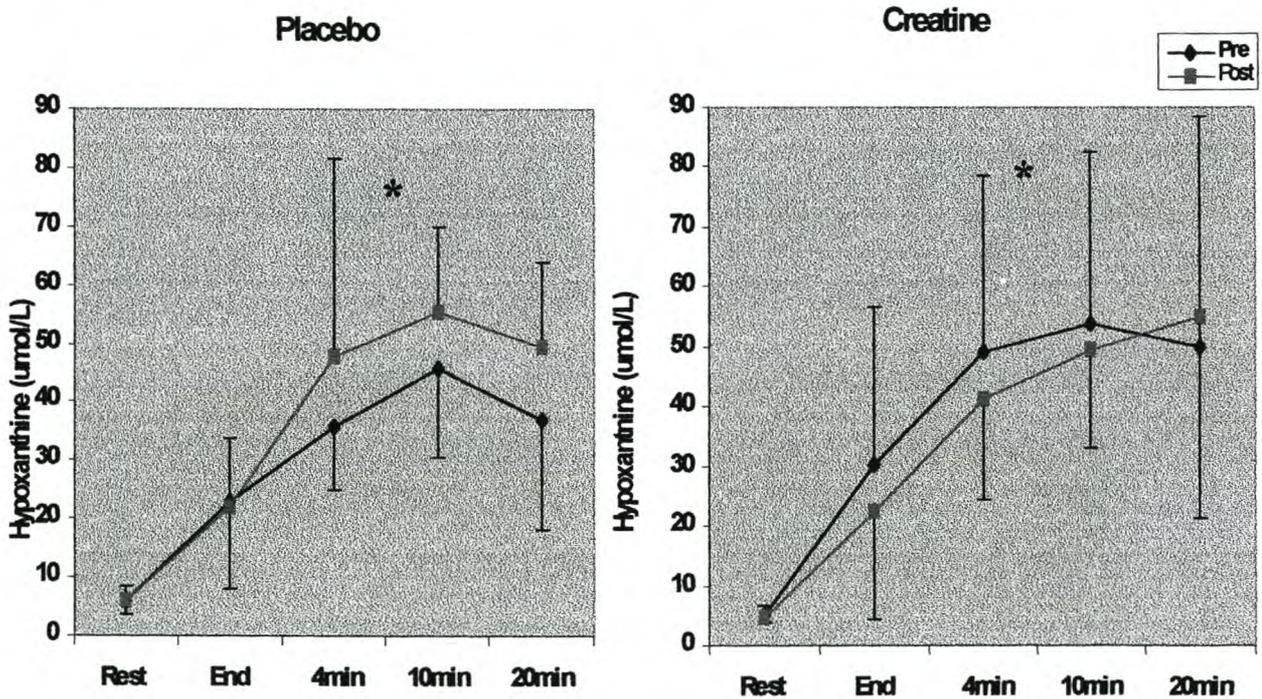
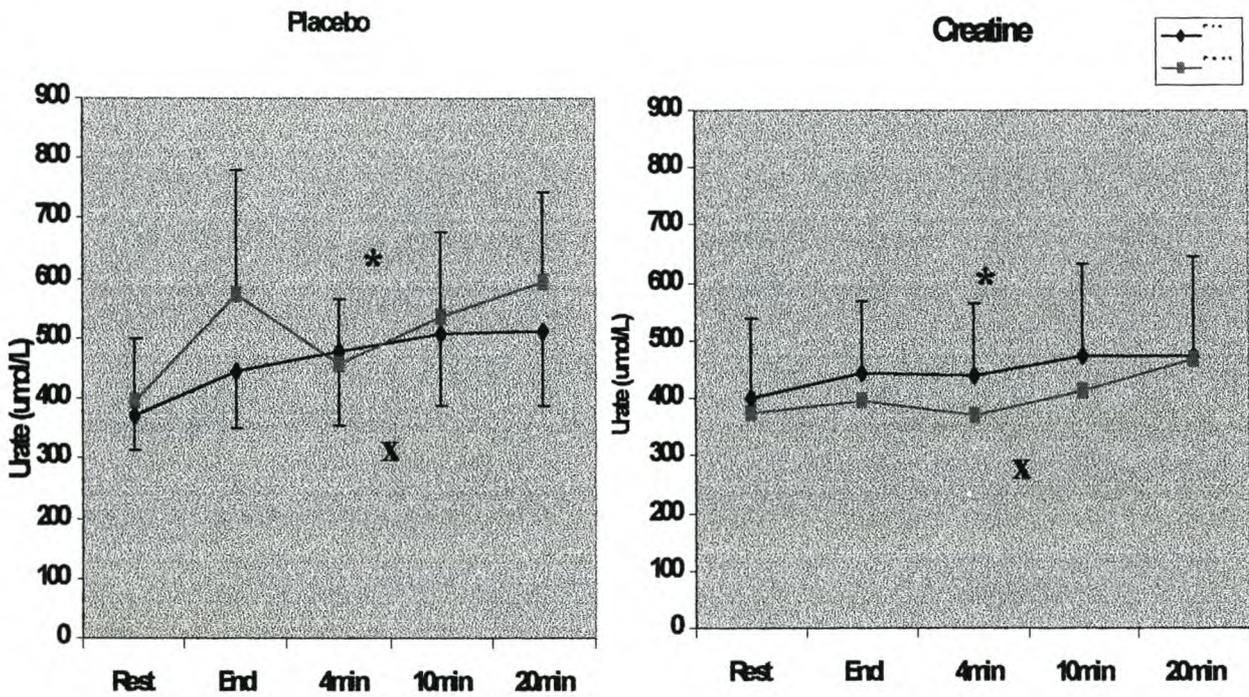


Figure 9: The comparison between plasma urate concentrations pre-and post the 7-day loading period in the placebo (n=6) and creatine groups (n=7). (Table of means \pm SD in Appendix 9)



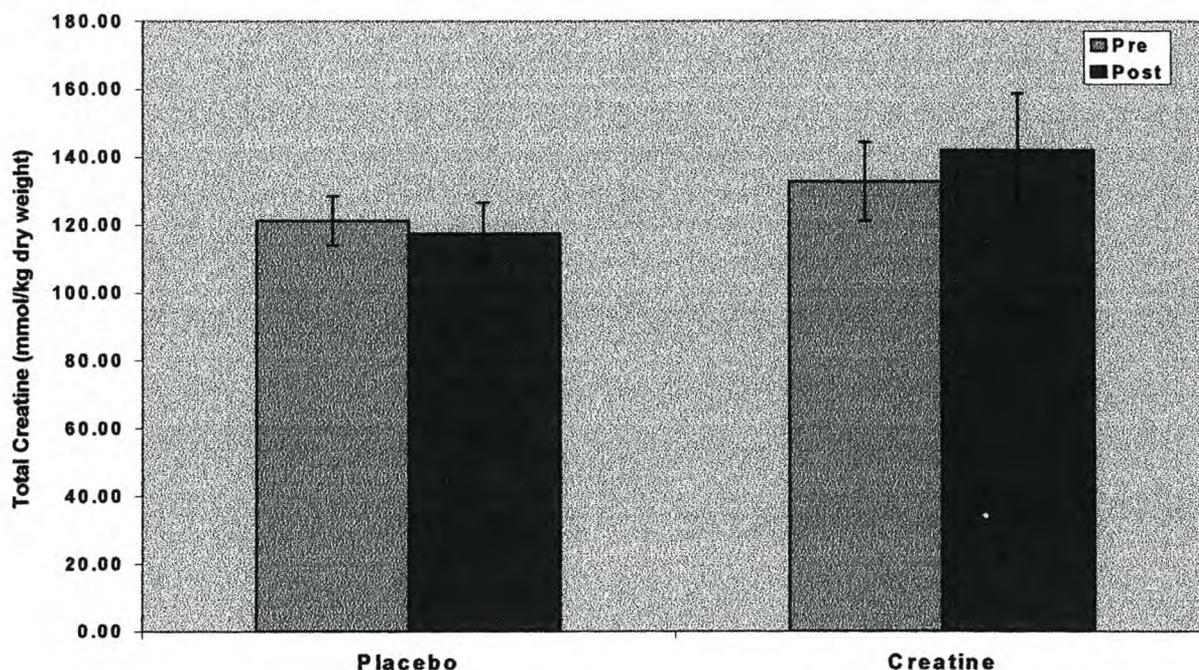
* = $P < 0.005$ (main effect for time)

x = $P < 0.005$ (group effect)

8.3.2 Muscle data

Muscle was analyzed in this study to determine total intramuscular creatine concentration and total intramuscular nucleotide concentration between the placebo and creatine groups. The creatine group showed a non-significant increase (7%) in mean total intramuscular creatine concentration.

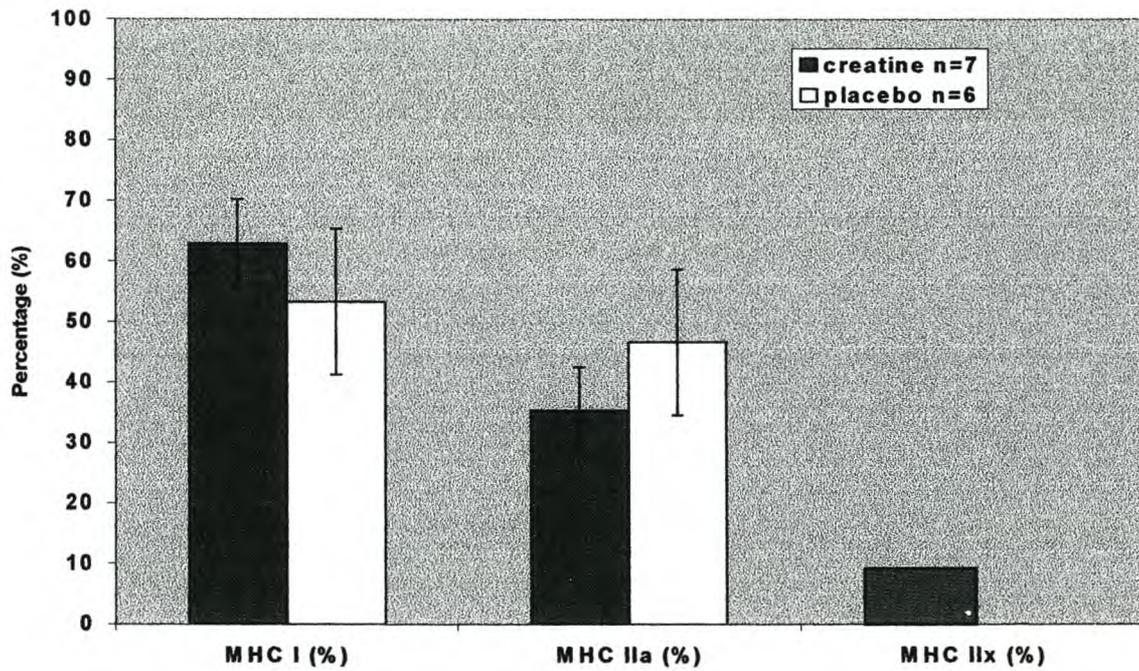
Figure 10: Intramuscular total creatine concentration (mean and SD) in both the placebo (n=6) and creatine groups (n=4) pre-and post supplementation. (Table of means \pm SD in Appendix 10).



Total intramuscular nucleotide concentration was also analyzed. No significant differences were found in the mean values for total nucleotide concentration (the sum of ATP and ADP as no AMP was found in the muscle analysis) pre- and post loading in the creatine and placebo groups. Creatine group values were 23.2 ± 6.1 mmol/kg dry wt pre-loading and 24.2 ± 2.7 mmol/kg dry wt post-loading. The placebo group showed a similar trend with 24.2 ± 2 mmol/kg dry wt pre-loading and 25.3 ± 1.9 mmol/kg dry wt, post loading.

The mean % MHC isoform distribution was also calculated for all the subjects in both the creatine (n=7) and placebo groups (n=6) (see Fig. 11)

FIGURE 11: The % MHC distribution between the creatine (n=7) and placebo groups (n=6). (Table of means \pm SD in Appendix 10).



CHAPTER 9: Discussion

9.1 Study 1

For road cycling the capacity to perform a “sustained sprint” is important during the race if an attempt is made to catch up with a pack of cyclists some distance ahead, or if a break away is attempted. However, “sustained sprint” capacity is not usually measured in the laboratory. We have used the 5 km TT as a measure of “sustained sprint” capacity and determined what factors may influence this capacity.

The following is a summary list of the major findings: i) There was a significant difference between STmean and TTmean due to the effect of prior fatiguing on subsequent exercise time trialing performance. ii) There was a good relationship between PSPO and 5 km time trial performance in the fresh (TTmean) as well as in the fatigued state (ST mean), although the relationship was statistically better in the fresh condition. iii) There was a statistically significant correlation between %MHC composition and plasma lactate concentration at time zero of recovery. The greater the %MHC I the higher the plasma lactate concentration at time zero.

Fatigue, induced by stochastic exercise definitely altered time trialing performance. All subjects (n=13) slowed their performance times between ST mean and TT mean with individual differences of 38 s at the top end of the scale to a single second as the smallest difference recorded. The fact that the subjects slowed down, was in itself an expected result. However, the extent that the subjects slowed down was so variable that we also determined whether this was related to any physiological variables, see later.

Only one other study that I am aware of has evaluated time trial performance following stochastic exercise. Palmer *et al.* (1997), evaluated the effects of stochastic versus steady-state exercise on subsequent time trialing performance. There are several aspects in which the study by Palmer *et al.* (1997) differed from the current study, making

comparison of actual results difficult. Firstly, in the study by Palmer *et al.* (1997) the time trialing distance was 20 km long and the stochastic test was a lot longer (150 min vs. 32min). Secondly, they did not evaluate the fatiguing effect of stochastic exercise relative to the fresh condition as I have done, but compared the fatiguing effect of stochastic exercise vs. steady state exercise of similar duration and average intensity on subsequent time trial performance. However, despite the similar work that was done in steady state prior to the time trial, they found that stochastic exercise was a more fatiguing form of exercise to do prior to a time trial.

The implications of doing laboratory testing that includes both fatiguing stochastic work and a time trial are that this type of testing, tests more than just “power” performance but also resistance to fatigue at various work intensities that may relate to various recruitment patterns. Therefore, possibly a more “complete” testing method. However we were unable to relate the extent of decrement in time trial performance following stochastic exercise to any physiological variable that we measured. It may be that not all the subjects performed similar amounts of high intensity work during training and that the design of the training programme (which I did not determine) is the main factor affecting the fatiguing effect of stochastic exercise. Stepto *et al.*, (1999), investigated the effect of varying the intensity of interval training on 40 km time trial performance in endurance cyclists. Their results showed that training that included several work bouts of 4 min duration at race pace resulted in the greatest enhancement of performance in a simulated 40 km time trial and also in the incremental test to determine peak power. It is therefore possible (even probable) that interval training would also affect time trialing performance after stochastic exercise.

In the current study the time trial distance was a lot shorter in length than those in the above mentioned studies. The 5-km time trial was extremely taxing on the cyclists as can be seen in the lactate levels produced (Fig. 2). This suggests that the energy for power production was not entirely supplied by the aerobic system in the 5 km time trial, but that the non-oxidative glycolytic system was used to a large extent. This type of exercise is often associated with high levels of hydrogen ion production (Neville *et al.*, 1989) and

therefore, performance may be affected by muscle buffering capacity, which we did not have enough muscle to measure. The addition of high intensity workloads as part of the fatiguing stochastic exercise, would also produce hydrogen ions and it is possible that the endogenous buffering capacity was already partly utilised prior to the ST time trial, thus affecting performance and explaining the decrement.

Another finding in the current study was the significant correlation between PSPO and TT mean and PSPO and ST mean. The higher the PSPO value, the faster the time trial performance time whether fatigued or not. The correlation between PSPO and TT mean was similar to the correlation that Hawley and Noakes, (1992) achieved for PSPO and 20km time trial speed were $r = -0.79$, $P < 0.01$ and $r = 0.68$, $P < 0.01$ respectively. A possible reason for the slightly better correlation between PSPO and 5-km time trial (fresh) than for the 20km time is that the PSPO test and the 5-km time trial were very similar in duration (time trial times were $7 \text{ min } 13 \pm 13.2 \text{ s}$ for TT mean compared to PSPO mean time for $n=13$ of $10 \text{ min } 26 \pm 1.5 \text{ s}$). It could be said that the metabolic requirements for both tests were fairly similar. If this is the case, it is perhaps not surprising that STmean showed the worst correlation with PSPO than did TTmean ($r = -0.60$, $P < 0.05$ and $r = -0.79$, $P < 0.01$ respectively). Both the power energy provision for STmean are different from those for TTmean, for example, within 40 minutes of cycling at $71 \pm 2 \%$ of VO_2peak (not even as intense as the average workload during stochastic test or 5 km TT) a cyclist can utilise 224 mmol/kg of glycogen Febbraio *et al.* (1998). Since glycogen utilisation rate decreases as muscle glycogen concentration decreases, the substrate for the last seven minutes of STmean would be substantially different from fresh TTmean Febbraio *et al.* (1998).

The subjects PSPO/kg ratio was correlated to time trial performance and no significant results were obtained. Therefore, it is more important to have a high muscle power per se, than to have a high muscle power relative to own body mass. This may however not always be the case for outdoor events. In the laboratory there is no component of wind resistance or gravitational force such as can be present outdoors and where body size per se could be a disadvantage. A surprising result was that the 105-km performance time

prediction was not at all related to any of the exercise performance variables (Table 2 in section 7.2.3). A possible similarity was hypothesised (prior to testing) between the time trial in the fatigued state (ST) and 105-km performance time. The 105-km mass start cycle race used as a criteria for the selection of the subjects also entailed variations in work intensities as was found by Palmer *et al.*, (1994). However the duration of the race was considerably longer (183.5 ± 11.3 min) than the laboratory test in this study (32 min + 7 min 32 sec ± 14.7 sec). The implication of this finding is that short duration laboratory tests can seldom be relied on to predict performance in races of longer duration.

There were significant changes in the plasma lactate concentrations from rest to exercise that lasted throughout the twenty minutes of recovery recorded in this experiment. The most interesting factor is that the mean plasma lactate recorded 20 minutes after exercise had ceased, was still 9.09 ± 2.02 mmol/L indicating that all subjects had failed to return to a value even close to an expected resting value. We hypothesise that lactate release from the muscle was prolonged. A possible explanation for our finding is that it is relative to that typically seen after an incremental PSPO test to fatigue. There plasma lactate concentration has an exponential decay rate after peak concentrations are reached approximately two minutes post-exercise due to saturation of the transport proteins, monocarboxylate transporters 1 and 4 (MCT 1 and MCT 4) (Bonen *et al.*, 1997). Muscle oxidative capacity was unrelated to plasma lactate disappearance rate during recovery. Therefore, we conclude that prolonged high levels of muscle lactate release overshadowed any influence of plasma lactate disappearance and subsequent oxidation on plasma lactate concentration itself.

In the current study plasma concentrations of hypoxanthine and urate increased during maximal exercise and recovery which is consistent with previous literature (Patterson *et al.*, 1982, Hellsten-Westing *et al.*, 1989; Strathis *et al.*, 1994). The increase in plasma hypoxanthine (precursor of xanthine and urate) during exercise and the further continuous increase during recovery is consistent with previous findings after maximal short-term exercise (Sollevi 1986; Patterson *et al.*, 1982; Hellsten-Westing *et al.*, 1989). In a different study, the relative increase in xanthine (3-fold) and urate (10%) were much

less than for hypoxanthine (27-fold) (Sahlin *et al.*, 1991). The interesting observation in the present study was that the highest mean concentration of hypoxanthine was at the 10 minute time point after termination of the exercise with a peak value of approximately 50 $\mu\text{mol/L}$. This is almost double the mean value obtained by Sahlin *et al.* (1991), who reported a peak value of $24.2 \pm 2.2 \mu\text{mol/L}$ attained 10 minutes after exercise into recovery. Their subjects cycled at a power output calculated to elicit 40% and 70% of VO_2max for 6-7 minutes each, where after the power output was increased to that calculated to correspond to 100% VO_2max , this exercise intensity being maintained to fatigue. The continuous response may be a consequence of the low activities of 5'-nucleotidase and nucleotide phosphorylase, which catalyse the transformation of IMP to inosine and from to inosine to hypoxanthine, respectively (Sahlin and Katz, 1988) so that conversion is saturated easily during exercise and must therefore continue after exercise to clear IMP and is still high after 20 minutes. The area under the curve calculated for hypoxanthine was not related to TT2, but was related to %MHC IIa ($r = 0.56$, $P < 0.05$) thus we can conclude that fast twitch fibres are accumulating more ADP or maybe have more AMP deaminase to convert AMP to IMP and subsequently to hypoxanthine.

It has been shown in previous studies that the decrease in the adenine nucleotide pool during maximal maximal exercise is about 15% and that a corresponding increase in IMP occurs (Sahlin *et al.*, 1991). In other studies arteriovenous differences across the previously exercised muscle of xanthine and urate are not significantly different from zero and imply that oxidation of hypoxanthine occurred in other tissues than the working muscle (Hellsten-Westling *et al.*, 1989; Strathis *et al.*, 1994). In summary, a 5 km TT, firstly produces substantial hypoxanthine and urate relative to other fatiguing exercise. Secondly, it results in prolonged high post-exercise hypoxanthine concentration, which are, thirdly related to % MHC IIa.

Muscle MHC composition also had a significant effect on plasma lactate concentrations. Immediate post-exercise lactate concentration correlated % MHC I ($r=0.59$, $P < 0.05$) and % MHC IIa ($r= -0.61$, $P < 0.05$). The correlation between lactate at time zero of recovery was positive with type I, so those with the highest slow twitch fibre ended up with the

highest lactate concentration at the end of the exercise. This is unlikely to be related to lactate production by the muscle fiber type itself because type IIa fibres have a higher capacity to produce lactate (Jensen-Urstad *et al.*, 1994). A possible alternative explanation for these results is that lactate transport capacity out of the working muscle is greater in type I than type IIa fibres. In support of this possibility is the data of Pilegaard *et al.* (1999) who showed that MCT 1 content correlates positively with % fibre type I. However, the issue is more complex than that, since MCT 1 can occur in the sarcolemmal and mitochondrial fractions (Dubouchaud *et al.* 2000). Dubouchaud *et al.* (2000) have shown that lactate release by muscle working at 65% VO_2peak is correlated positively with MCT 1 and MCT 4 contents in the sarcolemma.

Correlation analyses suggest a relationship between histochemically assessed percentage fiber type area and the electrophoretically assessed MHC content in human limb musculature (Fry *et al.*, 1994). In this study the relationship between percentage fibre type and the MHC content did not match up completely. The sample size for this analysis was one and was done as a case study. However because of possible histochemical misclassification of some fibers (especially trained muscle) both techniques may be important in yielding information about fiber type composition and possible fiber type transformations.

9.2 Study 2

In this study we determined whether oral creatine supplementation would affect 5-km time trial performance in the fresh condition after stochastic testing as well as whether it would affect metabolism during and recovery after 5 km TT. Based on a similar creatine loading regime to that first described by Harris *et al.* (1992), the trials were conducted.

The exercise performed in this research project was of an extremely high intensity and continued for 7-8 minutes when just the 5-km time trial was conducted and 39-40 minutes when the stochastic test was conducted with the 5-km time trial. It is likely that energy was provided by several of the energy systems from the pathways involving the

use of high-energy phosphates, adenine nucleotide metabolism, the glycolytic pathway and the oxidative pathway. Most of the research on creatine and performance has focused on high intensity short duration exercise (<30 s) (Balsom *et al*, 1993; Greenhaff *et al*, 1994; Dawson *et al*, 1995; Prevost *et al*, 1997) as well as more prolonged high intensity exercise (>30 s <150 s) (Febbrario *et al*, 1995 and Jacobs *et al*, 1997). Aerobic (>150 s) studies and creatine supplementation have also been completed (Barnett *et al*, 1996; Bellinger *et al*, 2000; Godly and Yates, 1997). Studies on creatine supplementation and 5-km time trial performance had not been conducted, to my knowledge, to date.

The results that we achieved to test our hypothesis that creatine supplementation would improve time trial performance after stochastic exercise showed that both groups slightly improved in their 5-km performance times in the fatigued state (ST mean vs. ST3) but not statistically significantly. Therefore the hypothesis is rejected. Balsom *et al*. (1993b), proved that creatine supplementation does not enhance endurance exercise performance. They investigated the influence of creatine supplementation on performance during a supramaximal run on a motor-driven treadmill until exhaustion and an approximate 6km terrain run. They mentioned that a likely explanation for a decrease in performance could perhaps be explained by an increase in body mass, which might have been the case in this study, although body mass change would be more likely to affect running than cycling. As mass was not monitored in the current study it thus can not be related to the performance times. Within the placebo group four subjects showed improvements in performance times (subjects 3, 6, 8, 13) and two did not (subjects 10 and 12) with subject 10 being 19 seconds slower. It could have been that the subjects of both groups were becoming more trained as the study continued and thus there were no significant differences in the data. The variation could also have been because the subjects thought that they might have been on creatine rather than placebo and therefore tried harder. Within the creatine group three subjects improved their performance times (subjects 1, 2 and 4) and four subjects marginally decreased their performance times (subjects 5,7,9, and 11). Therefore our study confirms that exercise performance with a large aerobic component is not affected by creatine supplementation (Stroud *et al.*; 1994, Bellinger *et al*. 2000). Our results suggest that 5 km TT does greatly affect adenine nucleotide

metabolism in the baseline, unloaded condition. In both tests TT2 and TT3 the hypoxanthine levels increased over time (Stathis *et al.*, 1994) and were at high levels during recovery and remained so, even 20-min into recovery time, an indication of the intensity that the 5-km time trial exerted on adenine nucleotide degradation (Fig. 8).

Another finding in this study was that the creatine loading had no effect on the alteration of the metabolism of plasma hypoxanthine and urate when comparing pre vs. post loading and the group effect. This is in contrast to what has been shown by Balsom *et al.*, (1993); Greenhaff *et al.*, (1993) in short term exercise and Bellinger *et al.* (2000) who found that plasma hypoxanthine was reduced ($P < 0.01$) in the creatine group over time in the one hour cycle test after loading. One difference from the current study is that they did have a larger sample group ($n=21$). A possible reason for a non significant result for hypoxanthine could be the large standard deviations. The slight alteration in metabolism did not improve the 5-km time trialing performance.

With the increase in plasma hypoxanthine there is the concurrent release and increase in plasma urate. The concentrations of urate did not increase much in comparison to that of hypoxanthine, but the values of urate remained high throughout recovery and plateaued and remained so even at minute 20 of recovery (Fig. 9). This study showed a significant difference between the concentrations at the different time points. There was also a significant difference between the groups in the pre-and post tests. In the placebo group the plasma urate concentrations during TT3 were significantly higher than during TT2. In the creatine group the mean values of urate during TT3 were significantly lower than during TT2 ($P < 0.005$). Thus creatine supplementation did have an effect on the plasma urate concentrations, which is in contrast to what Bellinger *et al.* (2000), found due, possibly, to the complex kinetics between muscle hypoxanthine production and release and urate formation

Total intramuscular creatine concentration was also determined. Creatine supplementation as described by Harris *et al.*, (1992), showed an increase of 20% (30% of this increase being attributed to an elevation in CP storage) which has also been confirmed by Greenhaff *et al.*, (1993). In this study the creatine group showed a non-

significant increase of 7% in mean total intramuscular creatine concentration (Fig 10). There are several explanations for this non-significant loading effect. Firstly, the initial value of the creatine group was relatively high (132.76 mmol/kg/dry wt) and it has previously been shown that subjects with lower baseline values load more than subjects with higher baseline values (Harris *et al.*, 1992; Delanghe *et al.*, 1989). Secondly, we had a small sample size (n= 4). Such values possibly occurred due to the creatine group sample being made smaller as a result of samples having defrosted. However this unavoidable circumstance was not the only influence on the significance of the result since there were also two non-responders in the creatine group that was left. The highest total creatine value attained by one of the subjects was 165 mmol/kg dry wt which surpasses the maximal level thought to exist by Harris *et al.*, (1992), of 150-160 mmol/kg dry wt, but only by a very small margin.

The mean concentrations of total nucleotide in the rested muscle did not differ much after the loading week, with a less than a 1% mean increase in both the creatine and placebo groups. The above result is expected as the effect of creatine supplementation is not to affect ATP concentration itself but to indirectly affect potential energy for ATP buffering. Some individuals varied total nucleotide levels by more than 1% and therefore as was seen in the methods section, creatine levels were corrected to an equal nucleotide value for each individual.

Plasma lactate concentrations were not analysed in this study as previous studies have shown that these values can remain unchanged (Birch *et al* 1994; Odland *et al* 1994) following oral creatine supplementation. The fact that plasma lactate levels are unchanged implies that there has been no alteration in non-oxidative carbohydrate metabolism in response to Cr loading. Two studies have shown a decrease (Balsom *et al.*, 1993; Söderund *et al.*, 1994). This could be that the exercise duration was so short that one could influence the onset of glycolysis by having more PCr around, thus delaying even by 1-2 seconds the onset of lactate accumulation. If the exercise duration is longer than 10 seconds this effect would not occur. In contrast, one study has shown lactate values to increase with creatine loading (Greenhaff *et al.*, 1994). The greater lactate

concentrations could be explained by either higher body mass to carry over the exercise terrain or more work done in the time because of creatine supplementation delaying fatigue.

9.3 Conclusions

The performance of the cyclists was monitored for the stochastic tests plus time trials as well as time trials in the unfatigued condition. The results of this study indicate that these tests were highly repeatable. The performance tests also resulted in high values for lactate, hypoxanthine and urate, an indication of fatigue achieved in the performance tests or possibly an indication of the high extent of anaerobic energy provision. These data are of the first to show an indication of the saturable nature of lactate release by muscle and the finding lends itself to further investigation.

Nutritional intervention of daily oral creatine supplementation of 20g of creatine for a 7 day period did not improve performance significantly in the stochastic 5-km time trials or the individual 5-km time trials. The results of this study may have been affected by several factors. The small sample size which may have reduced the statistical power of this study. However, financial constraints limited the amount of subjects that could participate in the study. Technical problems were also encountered during the study. Unfortunately, some of the muscle samples were lost due to the unexpected failure of the ultra-freeze refrigerator. These samples had to be discarded on the basis that normal values for muscle metabolites were not measured and thus affected the individual values for creatine, creatine phosphate and the adenine nucleotides. This resulted in the loss of 3 creatine loading subjects' data from the muscle analyses. However given the really large lactate responses to the 5 km TT and the less dramatic hypoxanthine and urate responses under baseline conditions, it may not be advisable to repeat the creatine loading study.

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Appendices

Appendix 1

Information sheet.

Project title: The effect of creatine supplementation on time trial performance after stochastic exercise.

Masters student: Andrew de Pao (under the supervision of Prof. K.H. Myburgh.)

Basic Protocol:

Week 1: Peak power output test (PPO) on an electro-magnetically braked cycle ergometer (Mon/Tues)

1st baseline stochastic test with 5-km time trial directly afterwards. (Thurs/Fri)
(The stochastic test on an electro magnetically braked cycle ergometer and the time trial on a ergometer such as the Spintrainer on which own bicycle will be attached.)

Week 2: 2nd baseline stochastic test with 5-km time trial directly afterwards. (Mon)

1st baseline 5-km time trial. (Wed)

2nd baseline 5-km time trial. Blood samples to be taken. Muscle biopsy to be conducted 1-2 hours post exercise.(Fri)

Week 3: Creatine loading for a week and training to be carried on as per usual.

Week 4: Final stochastic test with 5-km time trial (Mon/Tues)

Final 5-km time trial. Blood samples to be taken. Muscle biopsy to be conducted

1-2 hours post exercise.

- A muscle biopsy is a small surgical technique, which will be performed by a medical doctor experienced in this technique. It will be performed under sterile conditions and training will not be affected for more than one or two days.
- Blood samples will be taken during and after the 5-km time trial under sterile conditions.
- A maintenance dosage of creatine will also be given to the subjects after the loading week. (maintenance dosage = another week of creatine)
- During the whole study there should be no alteration in your training regimen.
- Avoid heavy training the day before each test.

Contact details:

Exercise Physiology Laboratory 808 4564 or 808 3149 (Dr. Myburgh).

Andrew de Pao 082 714 3171 or 5544105

Muscle biopsies performed by Dr. J.C. Koenig 883 2564

Appendix 2

CONSENT FORM

Exercise Science Laboratory,
Department of Human and Animal Physiology,
University of Stellenbosch.

Project Title: The effects of oral creatine supplementation on time trialing performance after stochastic testing.

Statement of understanding:

I have read and understand the explanations of procedures below. I have had the opportunity to ask the investigator any questions and understand that I am free to withdraw from the tests at any time should I so choose.

I confirm that:

1. I have been invited to participate in the research project at the Physiology Department at the University of Stellenbosch.
2. It has been explained that:
 - 2.1) The aims of this study will be to determine: The effects of oral creatine supplementation on time trialing performance after stochastic testing.
 - 2.2) Informed consent is required for the following procedures as part of the methods followed in the project - opposite the description the relevant procedures are marked X.
 - 2.3) It is expected that this project would be completed on a total of 12 to 16 subjects
The project will ideally be completed over a period of 4 weeks for each subject.
There will be a total of at least 7 visits to the exercise laboratory per week.
Each visit will last 1 hour to 2 hours.

Procedure # Visits EXPLANATION OF PROCEDURE

<input type="checkbox"/>	Blood Drawing elbow		Blood samples will be withdrawn for chemical analysis. Each time 3-10 ml (1/2 to 2 teaspoons) of blood will be withdrawn with a syringe. A needle will be inserted into the elbow vein No more than samples per visit = ml (teaspoons)
X	forearm		A needle + catheter is inserted into a forearm vein, the needle is removed and the catheter remains. samples per visit=14 (5ml per sample)
<input type="checkbox"/>	fingerprick		The fingertip is cleaned with an alcohol swab, pricked and squeezed until for a drop of blood. During the test the finger will be pricked times.
<input type="checkbox"/>	VO ₂ max Testing cycling		This test is performed in the laboratory, whilst breathing via a face-mask. Maximal exercise intensity to exhaustion is required for accurate results. on a cycle ergometer
<input type="checkbox"/>	running		on a treadmill
X	Maximum workload PPO		This exercise test will be performed in the laboratory. Maximal exercise intensity to exhaustion is required for accurate results. Peak Power Output: a continuous incremental test on a cycle ergometer
<input type="checkbox"/>	PTS		Peak Treadmill Speed test: a continuous incremental test on a treadmill
<input type="checkbox"/>	Wingate test		This test is performed at Human Movement Science on a sprint-cycle ergometer. Max sprinting speed is required for the duration of the test
<input type="checkbox"/>	Submax Ex steady state		Test will be performed in the laboratory on a cycle ergometer or treadmill. Set workloads will be completed for the required amount of time.
<input type="checkbox"/>	to fatigue		The set submaximal workload will be continued until exhaustion.
<input type="checkbox"/>	Intermittent Exercise test		This type of exercise intersperses bouts of low intensity and high intensity exercise at set percentages of maximum. As many repetitions as possible is required
X	training		The set number of intervals must be performed (12 intervals)
X	Time Trial		Test will be performed in the laboratory on the subject's own bicycle mounted on a spin-trainer. The rear inner tyre of the bicycle should be confirmed by the subject to be in a good condition.
<input type="checkbox"/>	Field test		This test will take place as a race under normal racing conditions.
<input type="checkbox"/>	Hypoxic test		During this test I will breathe a mixture of air containing 14% oxygen. This is 2/3 the amount usually present and is likely to make exercise more difficult.
<input type="checkbox"/>	Specialised test		Dexamethasone
X	Muscle biopsy		A small muscle sample (50 mg) for microscopic and chemical analysis will be taken. A medical doctor will perform this minor surgical procedure after administration of a local anaesthetic. To minimize potential infections (2% incidence; easily treated with antibiotics), it will be performed under sterile conditions. The subject must keep dressing in place for 3 days afterwards. Number of biopsies per visit: 1Total: 2

I also confirm that:

3. I have been informed of any possible side effects, discomfort or detrimental effects of participating in this study.
4. All the possible advantages of the study have been explained.
5. Information gathered in this study will be confidential. No names will be associated with individual results since every subject will be represented by a number unknown to them. The results will be used for a scientific assignment, publication or thesis, or all of these.
6. Results will be made available regarding the complete project as well as my own individual results once the project is completed.
7. I am free to withdraw from the study or any procedure at any time and that the researcher has the authority to remove me from the project, without impeding any participation at the relevant department in any other activity.
8. The information was presented in a language that I can understand and that I had the opportunity to ask any questions and that my questions were answered.
9. My participation was of my own choice without any pressure from the researcher / the department and that I am allowed to withdraw at any given time.
10. My participation will not involve any personal monetary costs.
11. I am currently healthy and exercise regularly with no adverse symptoms
12. If I had a muscle biopsy, I can contact Dr J.C. Koenig at tel: (021) 883 2564 at any time during the week following the biopsy.

I, _____ am _____ years of age and hereby give consent to undergo all of the above procedures according to the explained protocol.

Signatures:

Subject: _____ ID _____

Date: _____

Address: _____

Witness: _____ ID _____ Date _____

I, _____ declare that

1. The information in this document has been explained to: _____
2. That he was given the opportunity to ask questions and/ or to clarify any problems.
3. That the conversation was completed in English / Afrikaans without the need of an interpreter.

Signed at : _____ on _____

Signature: _____ Witness: _____

Exercise Science Laboratory,
Department of Human and Animal Physiology,
University of Stellenbosch.

Project Title: The effect of oral creatine supplementation on time trialing performance after stochastic testing

Statement of understanding:

1. I have read and signed the attached informed consent.
2. I understand that in addition to the results of the research, I will receive:

Tick the applicable box in the left-hand side of the table:

	Money	Product/ Procedure/ Value	EXPLANATION
<input type="checkbox"/>	<input type="text"/>		This amount will be paid if I have to take public transport to reach the lab and return home
<input checked="" type="checkbox"/>	R 200-00		This amount will be paid to compensate for the time I have invested in the project, but will only be paid if I complete the whole project and do not drop out of any of the tests or procedures.
<input checked="" type="checkbox"/>		Creatine	I will receive this product as part of the experiment or, if I received placebo, I will be given the active product at the end of the trial to take at home.
<input type="checkbox"/>			I will receive this procedure in addition to those that are part of the experiment to compensate me for the time I have invested in the project.

Signatures:

Subject: _____ ID _____

Date

Witness: _____ ID _____

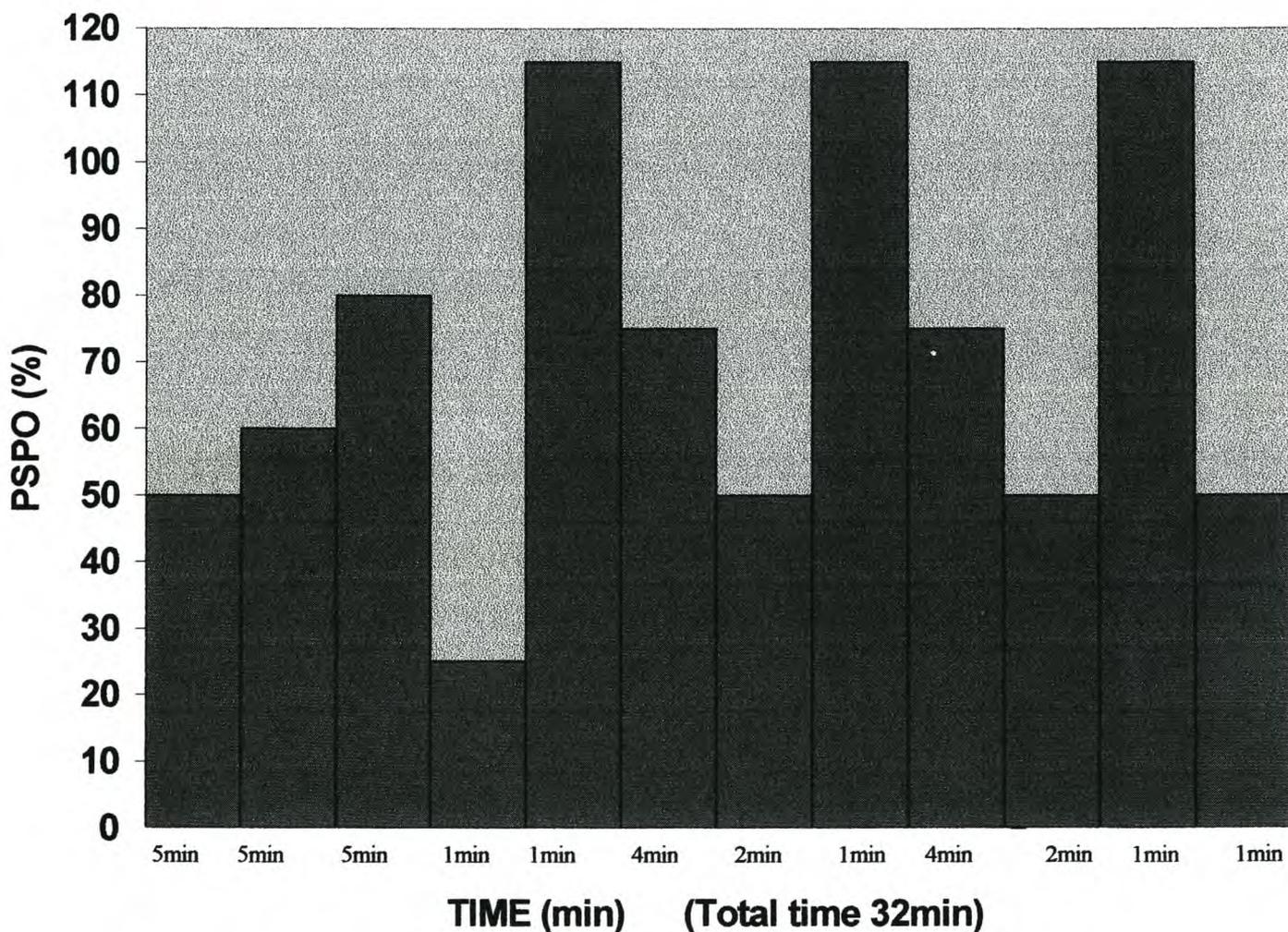
Date

Appendix 3.

The variable fixed-workload protocol (stochastic test) was as follows:

A warm-up of 5-8 minutes at 100 Watts. The stochastic test is graphically represented below with the amount of time spent on each workload (x-axis) and the percentage of PSPO (y-axis).

STOCHASTIC TEST



On completion of the 32 minute stochastic test the subjects performed a 5 km time trial in the fastest possible time.

Appendix 4

Freezing tissue for histochemistry.

The following equipment is needed:

Approximately 1-litre of liquid nitrogen
Isopentane
Tissue Tek® mounting compound
One pair of long-handed tongs
Scalpel blade
One pair of forceps
One large thermos flask
One small metal container
Cork board
35 mm film canister
Cutting board
Shaved ice

The freezing procedure is as follows:

1. Place the liquid nitrogen in the thermos flask and isopentane in the metal container.
2. Slowly lower the metal container into the liquid nitrogen and allow the isopentane to freeze until there is a pool of unfrozen isopentane in the centre. This creates optimum freezing conditions.
3. Place the muscle tissue on the cork with the orientation of the fibres being vertical and apply the Tissue Tek® mounting compound around and on top of the tissue sample.
4. Now place the tissue upside down into the pool of isopentane for 30-seconds so that the muscle comes into contact with the isopentane first.
5. Place the frozen sample into a film canister with some shaved ice (to decrease the rate of dehydration upon storage) and store it at -80°C .

Appendix 5

Myosin ATPase staining procedure.

Firstly the solutions have to be made up.

Solution A: Veronal buffer pH 9.4

50 ml 0.1 M sodium barbitone

50 ml 0.18 M CaCl₂

150 ml distilled water

Adjust the pH to 9.4 with 0.1 M HCl or 0.1 M NaOH

Solution B: 0.2 M Acetate buffer

0.2 M acetic acid

0.2 M sodium acetate

For pH 4.3: 40 ml 0.2 M acetic acid

20 ml 0.2 M sodium acetate

For pH 4.6: 20 ml 0.2 M acetic acid

40 ml 0.2 M sodium acetate

Adjust the pH with 0.1 M HCl or 0.1 M NaOH

Solution C: Substrate incubation solution.

10 ml 0.1 M sodium barbitone

5 ml 0.18 M CaCl₂

35 ml distilled water

150 mg ATP (di-sodium salt)

Leave the substrate solution at 37°C to warm, then adjust to pH 9.4 with 0.1 M HCl or 0.1 M NaOH as required.

NOTE: The substrate solution must be made up fresh before use.

The staining procedure is as follows:

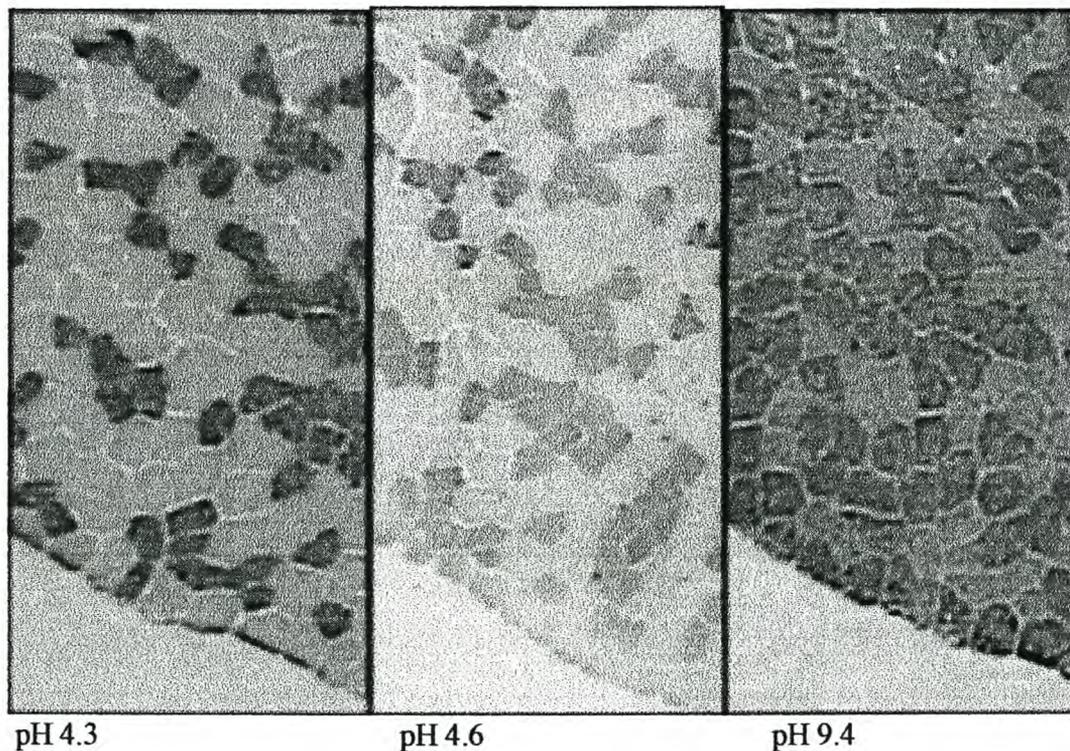
1. Pre-incubate the cryostat sections in the buffer of the appropriate pH for 15-minutes.
2. Take the sections from pH 4.3 and pH 4.6 buffers and rinse them in pH 9.4 buffer.
3. Incubate all the sections in pH 9.4 buffer for 5-minutes.
4. Now remove the sections from the pH 9.4 buffer and place them in the pre-warmed substrate solution at 37°C in a humid chamber for the appropriate times: pH 9.4 for 8-min, pH 4.6 for 12-min and pH 4.3 for 24-min. It is important to shake every 5-min to prevent CaCl₂ precipitates from producing uneven staining.
5. Wash well in 0.09 M CaCl₂ for 3-minutes.
6. Place in 2 % cobalt chloride for 3-minutes.
7. Wash well in 0.01 M sodium barbitone solution.

8. Rinse in distilled water and collect in a slide rack.
9. Develop colour on the slides using a dilute (approximately 1 %) ammonium sulphide in a fume cupboard for 30-seconds.
10. Wash in running water for 10-minutes.
11. Counter stain only the pH4.3 sections in eosin.
12. Rinse thoroughly in tap water.
13. Dehydrate the slides with ethanol of different percentages, 70 %, 90 % and 96 % in that order.
14. Clear the slides in xylol
15. The slides are now mounted with mounting gel.

The results will show the following:

Fibre	pH 9.4	pH 4.6	pH 4.3
Type I	Light grey	Dark grey	Grey/black
Type IIA	Dark grey/black	Light grey	Pink
Type IIB	Dark grey/black	Dark grey/black	Pink
Type IIC	Dark grey/black	Dark grey/black	Grey

The fiber typing results for one of the subjects was as follows at the various pH levels:



Appendix 6

Muscle MHC separation using Sodium Dodecyl Sulphate (SDS)-polyacrylamide gels.

The SDS-gels consisted of two gels, a separating gel and a loading gel as well as a denaturation buffer that were made up of different reagents.

The separating gel consisted of the following:

8 % acrylamide (w/v)
0.08 % bis-acrylamide (w/v)
0.4 % SDS, 0.2 mM
Tris-base (pH 8.8) ,and
27.5 % glycerol.

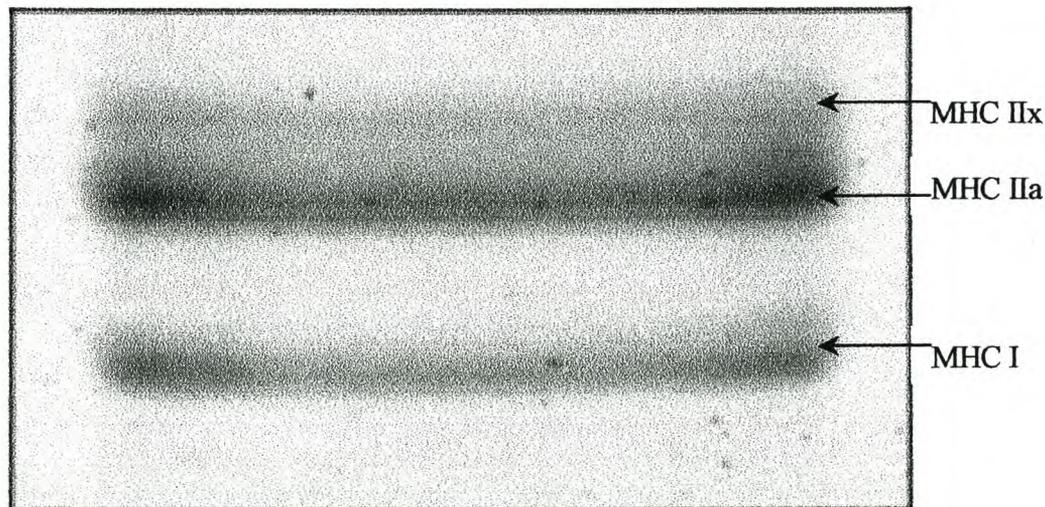
The loading gel consisted of the following:

3 % acrylamide (w/v)
0.16 % bis-acrylamide (w/v)
0.1 % SDS
125 mM Tris-base (pH 6.8).

The denaturation buffer consisted of

10 % glycerol (w/v)
5 % β -mercaptoethanol (v/v)
2.3 % SDS (w/v)
62.5 mM Tris-HCl (pH 6.8) and
0.02 % bromophenol blue and heated for 10 minutes at 60°C.

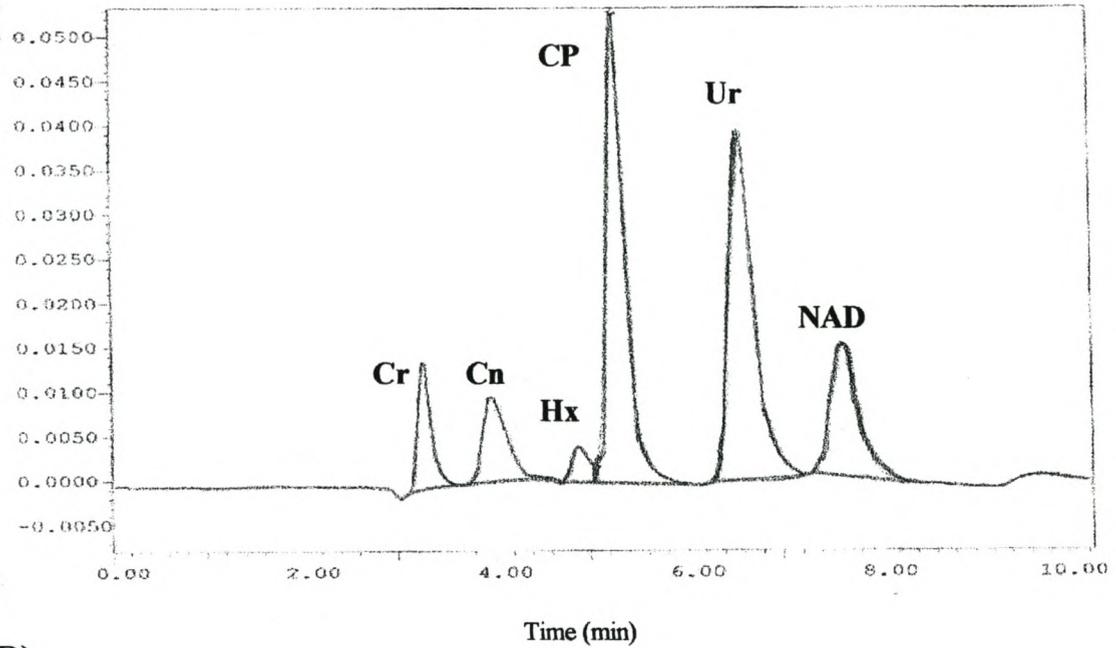
An example of the separation of the 3 MHC isoforms found in human skeletal muscles:



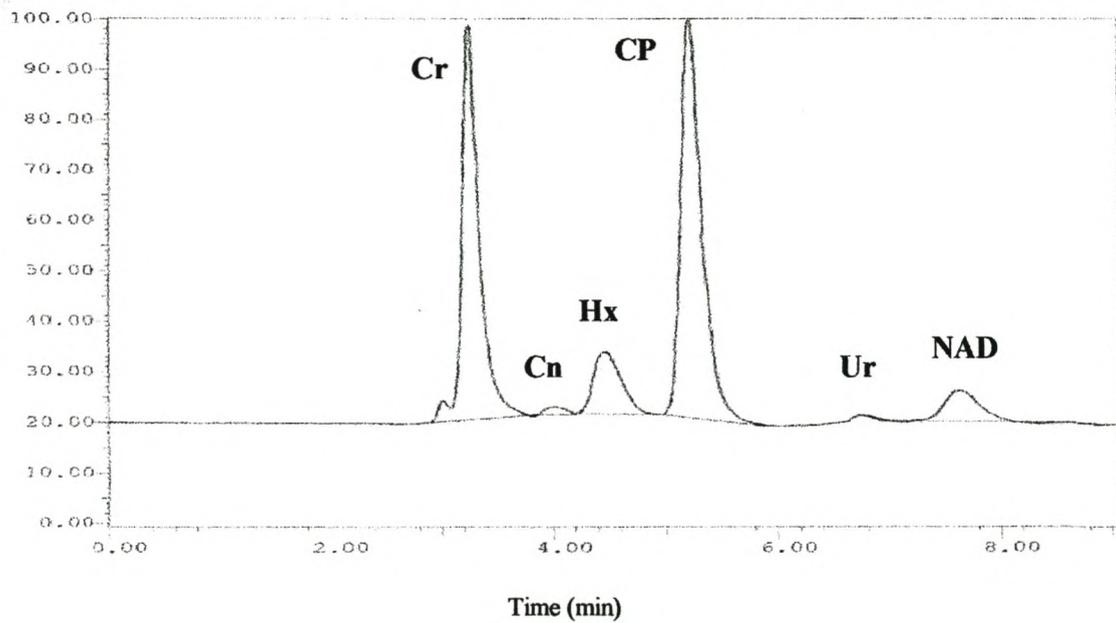
Appendix 7

An example of a chromatogram for both A) a muscle standard solution and B) muscle sample at 210nm

A)

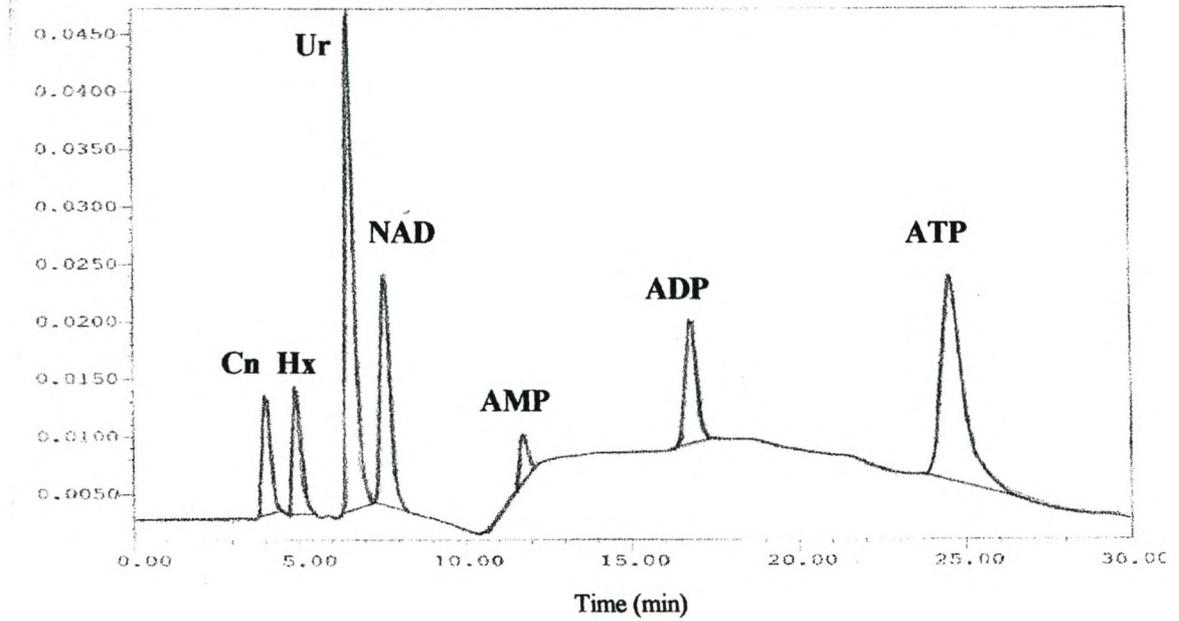


B)

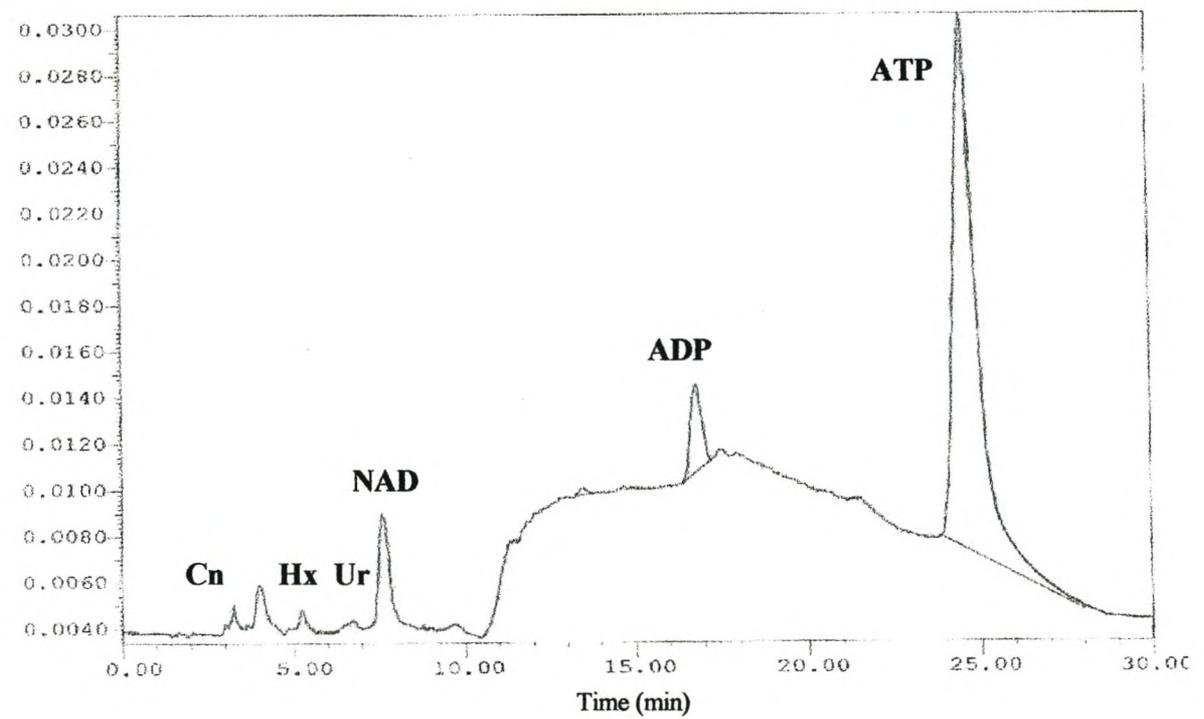


An example of a chromatogram for both A) a muscle standard solution and B) muscle sample at 249nm

A)

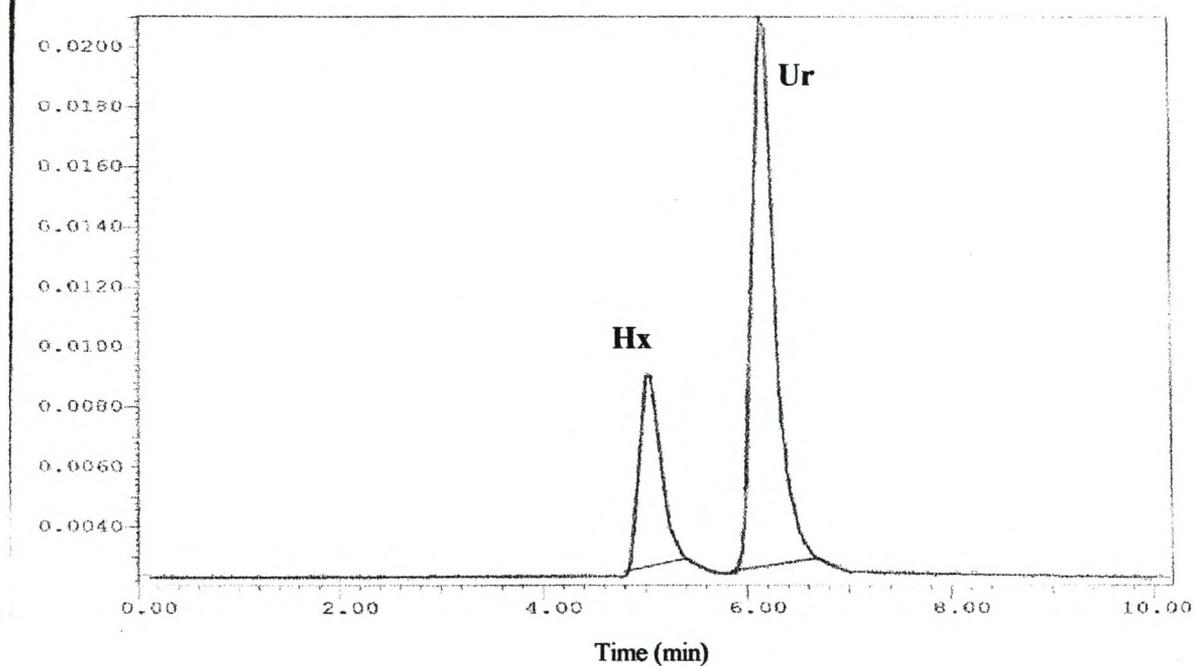


B)

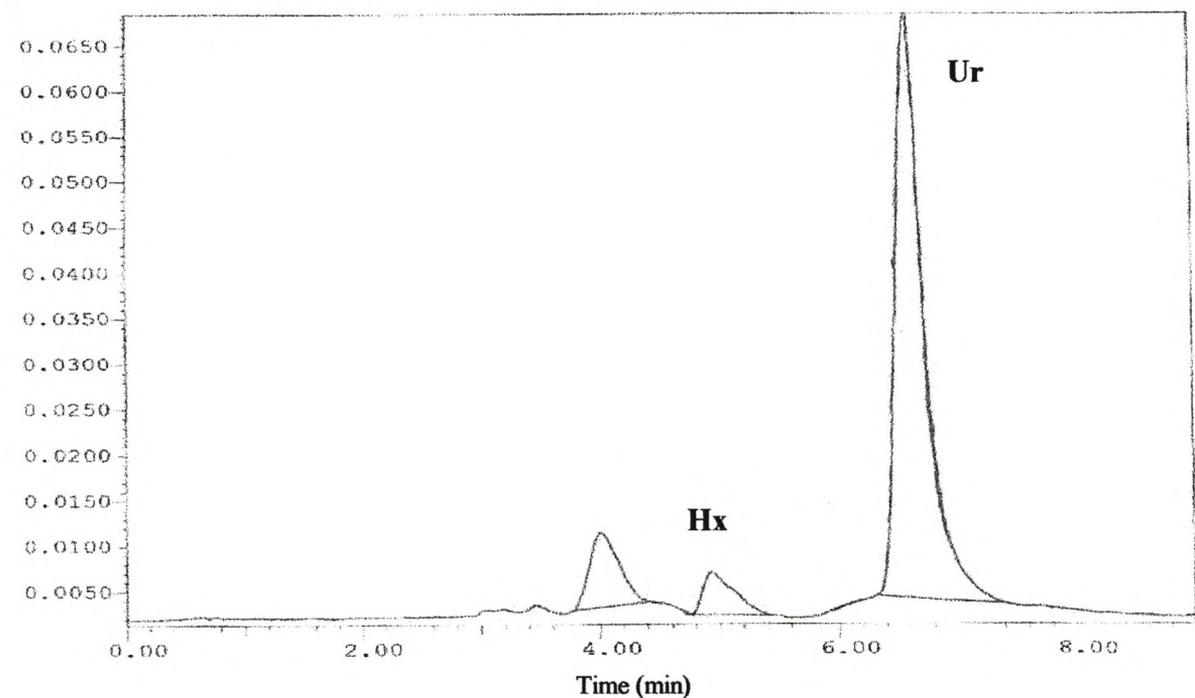


An example of a chromatogram for both A) a plasma standard solution and B) plasma sample at 249nm

A)



B)



Appendix 8

The table of means \pm SD for the 5 km time trials in the fatigued state (ST) and the individual 5 km time trial (TT). (Fig. 1)

Subject	ST1	ST2	ST mean	TT1	TT2	TT mean
1	7:42.0	7:32.0	7:37.0	7:20.0	7:28.0	7:24.0
2	7:42.0	7:38.0	7:40.0	7:04.0	7:00.0	7:02.0
3	7:24.0	7:29.0	7:26.5	7:24.0	7:27.0	7:25.5
4	7:12.0	7:08.0	7:10.0	7:03.0	7:07.0	7:05.0
5	8:00.0	7:54.0	7:57.0	7:28.0	7:34.0	7:31.0
6	7:20.0	7:15.0	7:17.5	6:52.0	6:58.0	6:55.0
7	7:44.0	7:34.0	7:39.0	7:02.0	7:08.0	7:05.0
8	7:21.0	7:18.0	7:19.5	7:00.0	7:03.0	7:01.5
9	7:28.0	7:23.0	7:25.5	7:04.0	7:04.0	7:04.0
10	7:24.0	7:26.0	7:25.0	7:21.0	7:26.0	7:23.5
11	7:33.0	7:26.0	7:29.5	7:15.0	7:15.0	7:15.0
12	8:00.0	7:54.0	7:57.0	7:34.0	7:33.0	7:33.0
13				7:40.0	7:44.0	7:42.0
mean	7:34.2	7:29.7	7:32.0	7:14.4	7:17.5	7:15.9
SD	00:15.6	00:14.0	00:14.7	00:14.7	00:15.2	00:15.0

The table of means \pm SD for the individual 5 km time trial (TT mean and TT3) performances before and after the loading week. (Fig. 6 and Fig. 7)

Group	TT mean	TT 3	ST mean	ST 3
Creatine mean	7:12.3	7:13.1	7:33.5	7:23.5
SD	00:11.4	00:13.8	00:15.9	00:10.7
Placebo mean	7:20.2	7:21.2	7:29.1	7:22.8
SD	00:18.3	00:24.3	00:16.0	00:25.4

Appendix 9

The table of means \pm SD for plasma lactate concentrations taken at TT2. (Fig.2)

	Rest	3tt	Time 0	2min	4min	7min	10min	20min
mean	1.48	7.68	12.91	13.46	13.27	12.48	11.07	9.09
SD	0.70	3.83	1.79	2.07	2.48	2.49	3.00	2.82

The table of means \pm SD for plasma hypoxanthine concentrations taken for TT2 and TT3 (pre and post loading). (Fig. 3)

Group	Rest	Time 0	4min	10min	20min
CreatineTT2	5.17	30.33	48.97	53.87	49.85
SD	1.60	26.44	29.78	28.56	38.61
TT3	4.57	22.28	41.31	49.62	54.99
SD	0.71	18.10	17.11	16.58	33.80
PlaceboTT2	6.04	22.82	35.71	45.67	36.82
SD	2.45	14.76	10.82	15.03	18.87
TT3	6.15	22.02	47.87	55.36	49.60
SD	2.32	11.61	33.55	14.41	14.26

The table of means \pm SD for plasma urate concentrations taken for TT2 and TT3 (pre and post loading). (Fig. 4)

Group	Rest	Time 0	4min	10min	20min
CreatineTT2	402.43	442.21	438.93	471.60	474.65
SD	134.69	127.28	123.05	160.82	170.59
TT3	373.98	397.06	370.18	414.93	471.17
SD	169.86	115.75	92.42	128.93	172.42
PlaceboTT2	369.84	444.60	479.54	508.94	511.55
SD	58.05	92.70	126.39	122.36	122.74
TT3	396.85	572.53	459.65	535.67	592.71
SD	101.21	208.85	107.40	142.28	149.02

Appendix 10

The table of means \pm SD for Creatine, creatine phosphate a Total creatine concentrations pre and post loading. (Fig. 10).

Group		Creatine	Creatine phosphate	Total creatine
Creatine	Pre	56.20	76.56	132.76
	SD	6.26	6.93	11.58
	Post	58.07	83.93	142.05
	SD	7.94	10.25	16.66
Placebo	Pre	50.42	70.78	121.20
	SD	5.69	8.44	7.27
	Post	48.74	68.74	117.48
	SD	9.28	10.48	9.06

The table of means \pm SD for total nucleotide contents pre and post loading. (Fig. 11).

Group		ADP	ATP	Total nucleotides
Creatine	Pre	1.59	18.25	23.19
	SD	0.76	9.11	6.05
	Post	1.71	18.96	24.20
	SD	0.73	8.71	2.72
Placebo	Pre	2.03	22.16	24.18
	SD	0.18	1.95	1.99
	Post	2.10	23.20	25.30
	SD	0.22	1.75	1.89

The table of means \pm SD for % MHC distribution in the vastus lateralis. (Fig. 12).

Group		%MHC I	%MHC IIa	%MHC IIx
Creatine	mean	62.97	35.35	1.67
	SD	7.27	7.13	4.41
Placebo	mean	53.34	46.66	0.0
	SD	12.05	12.00	0.0