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RECOVERY OF POWER OUTPUT AND MUSCLE METABOLISM FOLLOWING MAXIMAL SPRINT CYCLING IN HUMANS

by

Gregory C. Bogdanis

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of the Loughborough University of Technology

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ABSTRACT

The aim of the studies described in this thesis was to investigate the recovery of power output in relation to muscle metabolite changes, after maximal sprint cycling exercise.

During recovery from a maximal 30 s sprint (sprint 1), phosphocreatine (PCr) was resynthesised following an exponential time course with a mean half time of 56.6±7.3 s. Restoration of peak power output (sprint 2) occurred in parallel with PCr resynthesis, despite muscle pH remaining low throughout recovery (=6.7). However, neither PCr nor power recovery were complete after 6 min of rest (85% and 90% of control values, respectively).

The high power output during the initial 10 s of both sprint 1 and sprint 2 was mainly supported by energy supply from PCr degradation (30-35%) and anaerobic glycolysis (42-49%). However, the contribution from PCr was diminished during the remaining 20 s of the sprint, due to the decreased PCr concentration, leaving glycolysis and aerobic metabolism as the main energy sources. As much as 65% of the total estimated energy was supplied by aerobic metabolism during the last 20 s of sprint 2. A high correlation was found between maximal oxygen uptake (VO2max) and the % aerobic contribution to the total estimated ATP utilisation during sprint 2 (r=0.87, P<0.01).

The significance of muscle blood flow during the recovery period as a common regulator of PCr and power output recovery, was examined by performing an “active” recovery (cycling at 40% VO2max) between two 30 s sprints. Active recovery improved power output restoration during the initial 10 s of sprint 2 (by 3.0±0.7%, P<0.05), while the ability to accelerate (as reflected in the work done to accelerate the flywheel) was improved by ~8%. However, there was no difference in power output between “active” and “passive” recovery during the last 20 s of sprint 2. Although an improved PCr resynthesis after “active” recovery is an attractive explanation, a faster lactate and [H+] efflux from the muscle and an increased aerobic contribution (as shown by a 18% increase in VO2 during the 2nd sprint), may have also played a role.

The recovery of muscle metabolism and power output was also examined after sprints of different duration. A 30 s sprint (sprint 2) was performed on two separate occasions following either a 10 s or a 20 s sprint. PCr resynthesis 2 min after the 10 s sprint and the 20 s sprint was similar (86% and 76% of resting, n.s.), but muscle lactate was different (38 vs 66 mmol·kg dry muscle⁻¹). Restoration of peak power output was complete when sprint 1 was 10 s , in spite of the elevated muscle lactate. However, the mean power during the first 10 s of sprint 2 was 5% lower compared with sprint 1. Power output during sprint 2 after the 20 s sprint was lower compared with both sprint 1 (in the same experimental condition) and with sprint 2 when sprint 1 was 10 s.
During sprinting on a friction loaded ergometer pedal speed changes from the start to the end of a sprint. The effects of power-velocity relationship on power output during repeated sprints were assessed by sprinting against different resistive loads. A higher mean power was generated when sprinting against a heavy, compared with a light resistive load (mean pedal speed: ≈125 revs·min⁻¹ vs ≈175 revs·min⁻¹, respectively). During repeated 6 s sprints against resistive loads of 50 and 100 g·kg body mass⁻¹, the higher power output (heavy load) was not accompanied by a greater fatigue, and this was interpreted as an increased efficiency due to cycling closer to an "optimum pedal speed" of ≈120 revs·min⁻¹.

The studies presented in this thesis have provided evidence which suggests that power output is almost completely restored during the first few minutes of recovery after sprint exercise, despite the low muscle pH. Although glycolysis provides the major part of the energy during maximal sprinting, PCr plays a dominant role when a sprint is repeated. The significance of PCr lies in the fact that it can be rapidly resynthesised and therefore is a readily available energy source during the initial seconds of a repeated sprint. Furthermore, the PCr system is the fastest to respond to the ATPase activity and to buffer the ATP hydrolysis products (ADP and H⁺) which have been shown to be involved in fatigue.
Unless otherwise indicated by acknowledgements or references to published literature, the work contained herein is that of the author and has not previously submitted for another degree in this or any other University.

Part of the work contained in this thesis has been published as follows:

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...αν να κρατας καλα μπορεις το λογικο σου, οταν τριγυρω σου ολοι 
tαχουν χαμενα και σ' εσε της ταραχης των ριξουν την αιτια 
αν να εμπιστευεσαι μπορεις τον ιδιο τον εαυτο σου, οταν ο κοσμος δεν σε 
πιστευει, κι αν μπορεις να του συχωρνας αυτη την δυσπιστια 
να περιμενεις αν μπορεις διχας να χανεις την υπομονη σου 
κι αν αλλοι σε συκοφαντουν μην καταδεχθεις ποτε το θεμα 
κι αν σε μισου εσου ποτε σε μισος ταπεινο να μην ξεπεσεις, μα να μην 
κανεις τον καλο η τον πολυ σοφο στα λογα 
αν να ονειρευεσαι μπορεις και να μην εισαι δουλος των ονειρων 
αν να στοχαζεσαι μπορεις, διχας να γινει ο στοχασμος σκοπος σου 
αν να αντικριζεις σου βαστα το θριαμβο και τη συμφορα παρομοια, κι 
αν ομοια φερεσαι σ' αυτους τους δυο τυραννικους απατεπνευ 
αν σου βαστα η γυχη ν' ακους οποιαν αλθεια εσου ειχες επαμενη 
παραλλαγηνη απ τους κακους γα ναναι γα τους αμωλος παγιδα, η 
συντριμενα να θερεις οσα σου εχουν ρουφηξει τη ζωη σου και παλι να 
ξαναρχινας να χτιζεις μ' εργαλεια που ναι φθαρμενα 
αν οσα αποχτησες μπορεις σ' ενα σωρο μαζι να τα μαξεψεις και διχως 
φοβο, μονομιας κορονα η γραμματα ολα να τα παιξεις και να τα χασεις, 
κι απ αρχης απρανταχτος να ξεκινησεις παλι και να μην βγαλεις και 
μιλια ποτε γ' αυτο τον ξαφνικο χαμο σου 
αν νευρα και καρδια μπορεις και σπλαχνα και μυαλο και ολα να τα 
σφιξεις να σε δουλεψουν ξαναρχης, κι ας ειναι απο πολυ καιρο 
σκομμα 
και να κρατιεσαι παντα ορθος, οταν δεν σου χει τιποτε απομεινει παρα 
μοναχα η θεληση, κραζοντας σ' ολα αυτα: “βαστατε”. 
αν με τα πληθη να μιλας μπορεις και να κρατας την αρετη σου με 
βασιλιαδες να γυρνας διχας απ τους μικρους να ξεμακρυνεις 
αν μητε φιλοι μητ εχερι ιμπορουν πια ποτε να σε πειραξουν 
ολο τον κοσμο αγαπας, μα και ποτε παρα πολυ κανενα 
αν του θυμου σου της στιγμες, που φαινεται αδυσωπητη η γυχη σου, 
μπορεις ν αφησεις να διαβουν, την πρωτη ξαναθρισκοντας γαληνη, 
δικη σου θα ταν τοτε η γη, μ' οσα και μ' οτι απανω της κι αν εχε 
και κατι ακομα πιο πολυ: ανδρας αληθινος θα σαν παιδι μου. 

("αν", Rudyard Kipling, 1892)
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CHAPTER I

INTRODUCTION

For many years it has been recognised that the performance of muscular work is dependent on energy derived from chemical reactions within the tissue. The immediate energy substrate is ATP, and the challenge for the energy-delivering processes is to sustain a rate of ATP resynthesis that matches the rate of ATP utilisation. This challenge for the energy delivering processes is probably greater during sprint exercise where extremely high rates of ATP resynthesis are required. The major part of the energy for sprint exercise is provided by anaerobic glycolysis and phosphocreatine (PCr) breakdown (Nevill et al., 1989; Gaitanos et al., 1993). Thus accumulation of lactate and H⁺ and depletion of PCr occur early in the sprint (Boobis et al., 1982; Jacobs et al., 1983) and have both been considered as fatigue factors (Spriet et al., 1989; Boobis et al., 1987).

Most of the studies examining the interrelations between muscle metabolism and muscle function have used voluntary isometric exercise (e.g. Katz et al., 1986a; Sahlin and Ren, 1989) or electrical stimulation of the knee extensors (Hultman and Sjoholm, 1983; Soderlund et al., 1992). In an attempt to identify the causes of fatigue (which was manifested as a decrease in force) the concentration of important metabolites immediately after exercise has been correlated with the decrease in force. However, the fact that several metabolic changes occur almost simultaneously during intense contractions, made it very difficult to show that a single factor was responsible for fatigue (Hultman et al., 1990). Thus, the reduction of phosphocreatine (Sjoholm et al., 1983; Katz et al., 1986a) and muscle pH (Cady et al., 1989a) and the accumulation of inorganic phosphate (Wilson et al., 1988) have each been characterised as the main determinant of the loss of force during isometric exercise.

The use of sprint exercise as a tool for studying the effects of muscle metabolite changes on muscle function is very attractive. Measurement of power output incorporates both force and velocity components and represents a situation closer to real life, compared with intense isometric contractions and electrical stimulation methods. The development and
instrumentation of a sprint cycle ergometer by Lakomy (1986, 1988) has enabled accurate and 'high resolution' measurements of power, speed and acceleration during maximal sprinting. Unlike isokinetic cycle ergometers (where pedalling rate is kept constant), this ergometer allows the subject to accelerate and then slow down as fatigue occurs. At the same time, power and velocity are precisely monitored. The data acquired from power output measurements allow evaluation of various aspects of muscle function which are overlooked when 'static' measurements are made (e.g. effects of fatigue on the rate of muscle relaxation). Thus, the combination of the muscle biopsy technique and 'high resolution' measurements of power may further our understanding of the complex fatigue mechanisms. Additionally, the results obtained from these measurements are highly applicable to sports training and competition, because the mode and intensity of exercise resemble sports situations.

The results of the relatively few studies that have examined muscle metabolite changes during sprint exercise (e.g. Jones et al., 1985; Cheetham et al., 1986) show that it is difficult to separate the effects of each metabolite on power output when attempting to identify the causes of fatigue. Two different approaches can be followed in order to increase our understanding of fatigue during sprinting:

(i) the model of repeated exercise bouts is a potentially useful method to separate the effects of different metabolites on power output. For example, Gaitanos et al. (1993) has shown that during the last of ten 6 s sprints, separated by 30 s rest, anaerobic glycolysis was almost arrested and the muscle was dependent mainly on PCr degradation and aerobic metabolism. This study pointed to the significance of PCr availability during short repeated sprints, which is due to the ability of PCr to be rapidly resynthesised and be available for energy production (Harris et al., 1976), at times when glycolysis is still significantly impaired.

(ii) the second way to gain more information about the relationship between power output and metabolism during sprinting is to repeat sprints of different duration. The power output profiles obtained during sprinting, show that there is a marked decrease in power during the first few seconds of the sprint, followed by a slower decline. Preliminary results (Boobis et al., 1982; Jones et al., 1985) have suggested that the ATP utilisation is decreased rapidly from the first few to the last seconds of a 30 s sprint. It is therefore interesting to examine the relative contribution of
the energy supply mechanisms at the start and towards the end of single or repeated sprints.

The main aim of this thesis was to examine the relationship between muscle metabolites and power output recovery following a bout of maximal sprint exercise in order to contribute to furthering understanding of the aetiology of muscle fatigue. This thesis is presented in the eight following Chapters:

- The review of literature (Chapter II), sets the conceptual background for the studies that follow. The mechanisms of energy supply during sprinting are presented, and potential causes of fatigue are discussed. Special reference is made to the effects of the power-velocity relationship of human muscle on power output during sprint cycling.
- In the general methods (Chapter III) the equipment, methods of analysis and general procedures followed during testing are presented.
- The aim of the first study (Chapter IV) was to examine the recovery of important muscle metabolites (PCr, H+) in relation to power output restoration after a maximal 30 s sprint.
- The aim of the second study (Chapter V) was to examine muscle metabolism during the first 10 s and the last 20 s of a second (repeated after 4 min) 30 s sprint. This design provides useful information regarding the extent of the contribution of PCr and glycolysis at the start and towards the end of sprint 2.
- The third study (Chapter VI) investigated the effects of 'active' recovery between two 30 s sprints, in an attempt to examine the significance of muscle blood flow during the recovery period.
- The purpose of the fourth study (Chapter VII) was to examine recovery of muscle metabolites and power output following sprint exercise of differing durations (10 s and 20 s).
- Sprinting on a friction loaded ergometer results in a range of pedalling speeds attained from start to end of the sprint. This change in speed affects power output purely as a result of biomechanical (and not metabolic) factors. The fifth study (Chapter VIII) assessed the effects of power-velocity relationship on power output by sprinting against different resistive loads.
- The final chapter (Chapter IX) integrates the findings of the studies conducted for this thesis, and explains possible mechanisms involved in fatigue during maximal sprint exercise.
CHAPTER II

REVIEW OF LITERATURE

2.1. INTRODUCTION

Understanding of the regulation of energy metabolism during high intensity (sprint) exercise may provide some insight into the causes of skeletal muscle fatigue. During sprint exercise, muscle metabolism is stressed to near maximal levels, while changes in intramuscular metabolites are large. The aim of this review is to present a framework of the biochemical events associated with fatigue and recovery during maximal sprint exercise. This review has been divided into five major sections.

One of the major causes of fatigue is considered to be the failure of metabolism to provide energy at the rate required by working muscles. Therefore, the energy supply mechanisms and the metabolic profile of the working muscle during this type of exercise are discussed thoroughly (section 2.2). The fact that the main fuel for sprint exercise comes from anaerobic glycolysis results in large decreases in muscle pH. The changes in acid base balance in the muscle and blood are discussed in section 2.3. Since repeated bouts of exercise have been employed in the present study, the changes in muscle metabolites and power output during the recovery period after high intensity exercise are reviewed in section 2.4. Emphasis is placed on the resynthesis of phosphocreatine, recovery of force and power, and restoration of muscle pH. The role of muscle blood flow during recovery is also discussed. Section 2.5. examines the causes of skeletal muscle fatigue during high intensity exercise at the level of the muscle fibre. The review concludes by discussing the effects of force-velocity and power-velocity relationships of the muscle on power output generation during maximal sprint cycling (section 2.6).
2.2. ENERGY SUPPLY DURING HIGH INTENSITY EXERCISE

For the successful completion of any physical task, chemical energy must be efficiently converted into mechanical energy, at rates appropriate to the muscles' needs (Hultman and Harris, 1988).

During a maximal 30 s sprint, where the energy demands are more than 2.5 times greater than the aerobic power ($\text{VO}_2\text{max}$) can supply (Wootton and Williams, 1983), the muscle cell is dependent on the energy provision systems that result in the greatest rates of adenosine-5-triphosphate (ATP) resynthesis, namely the creatine kinase/phosphocreatine system (CK/PCr) and anaerobic glycolysis. It has been estimated that these energy systems can res synthesise ATP at a maximum rate of ≈8.6 (CK/PCr) and ≈6.0 (anaerobic glycolysis) mmol·kg dry muscle$^{-1}$·s$^{-1}$, and reach this power in <1 s and 5 s, respectively (Sahlin, 1986a). Aerobic metabolism is considered as a low power-high capacity system, which is fully activated after about 3 minutes of exercise (Sahlin, 1986a).

The estimated ATP utilisation rate from anaerobic sources during a 30 s treadmill sprint ($\frac{\text{ATP utilisation}}{30 \text{s}}$) is around 6.1-6.3 mmol·kg dry muscle$^{-1}$·s$^{-1}$ (Cheetham et al., 1986; Nevill et al., 1989). Similar values (6.2-7.9 mmol ATP·kg dry muscle$^{-1}$·s$^{-1}$) have also been calculated for a 30 s sprint on friction-loaded (Boobis et al., 1982, 1983) or isokinetic cycle ergometers (Jones et al., 1985; McCartney et al., 1986). When the duration of the sprint is shorter, the anaerobic ATP utilisation rate can be as high as ≈15 mmol·kg dry muscle$^{-1}$·s$^{-1}$ (6 s cycle ergometer sprint; Gaitanos et al., 1993). This is because the anaerobic ATP utilisation rate is high at the start of the sprint and drops considerably thereafter (Boobis et al., 1987).

The anaerobic ATP utilisation rate has been shown to be considerably reduced when subjects repeated a 30 s bout. Rates as low as 2.5 and 1.9 mmol·kg dry muscle$^{-1}$·s$^{-1}$, have been calculated during the second and third bout of isokinetic cycling at 100 revs.min$^{-1}$ (Spriet et al., 1989).

During isometric exercise (either voluntary contractions or electrical stimulation) the rate of anaerobic energy utilisation is lower compared with sprint exercise (Katz et al., 1986a; Spriet et al., 1987a; Chasiotis et al., 1987; Bergstrom and Hultman, 1988). For example, during intermittent electrical stimulation (at 20 Hz) of the quadriceps femoris muscle for 10 s (corresponding to ≈70% of the maximal voluntary contraction force), the anaerobic ATP utilisation rate was 6.9 mmol·kg dry muscle$^{-1}$·s$^{-1}$ (Chasiotis et al., 1987).
CHAPTER II: Review of Literature

This is about half of the rate of $=15$ mmol·kg dry muscle$^{-1}$·s$^{-1}$ reported by Gaitanos et al. (1993) for sprint exercise over a similar time period (6 s). Even during a single near-maximal stimulation (1.26 s electrical stimulation equivalent to $=95\%$ of maximal voluntary force) the anaerobic ATP utilisation rate (11 mmol·kg dry muscle$^{-1}$·s$^{-1}$) was still lower than the rates calculated (or expected) during sprint exercise (Hultman and Sjoholm, 1983). One possible explanation for the difference in energy utilisation between isometric (continuous or intermittent) and sprint cycling exercise may be that the number of contraction/relaxation cycles is much higher during sprinting, resulting in more ATP consumed for activation and relaxation of the muscle (Bergstrom and Hultman, 1988).

Therefore, it can be argued that sprint cycling exercise requires probably the highest rates of energy supply.

2.2.1. Role of ATP as the "energy currency" of the cell

The initiation of muscle contraction is associated with calcium release from the sarcoplasmic reticulum (Fig. 2.1). This release couples electrical, mechanical and biochemical events of contraction and the breakdown of ATP. Hydrolysis of ATP to ADP (adenosine diphosphate) and inorganic phosphate (Pi), provides the immediate energy for most energy-consuming processes in the cell:

$$\text{ATP} + \text{H}_2\text{O} \rightarrow^{\text{ATPase}} \text{ADP} + \text{Pi} + \text{H}^+ + \text{energy (7.3 kcal/mol)}$$

where Pi = inorganic phosphate

However, the amount of ATP stored in the muscle cells is very limited ($\approx 20-30$ mmol·kg dry muscle$^{-1}$), and would last only for a few contractions during maximal exercise (Hochachka and Matheson, 1992). It is therefore clear that ATP utilisation rates must be closely balanced with ATP resynthesis rates, if a particular exercise intensity (and the ATP store itself) is to be maintained. In that respect, it would seem more appropriate to regard ATP as a mediator of energy, rather than as an energy source (Sahlin et al., 1978b).

During the contraction-relaxation cycle, three different ATP splitting reactions, catalysed by 3 different isoenzymes (ATPases), can be distinguished:
Fig. 2.1. Simplified representation of the events leading to muscle contraction. Depolarisation spreading along the t-tubule system causes the sarcoplasmic reticulum to release $Ca^{2+}$ and thus stimulate myofibrillar ATPase. $Ca^{2+}$ ions are transported back into the sarcoplasmic reticulum at the expense of ATP hydrolysis (in Newsholme and Leech, 1983).

- actomyosin ATPase, for dissociation of cross-bridges (Jones and Round, 1990)
- calcium ($Ca^{2+}$) transport ATPase, for $Ca^{2+}$ reuptake by the sarcoplasmic reticulum (Klug and Tibbits, 1988; Carafoli, 1991), and
- $Na^+\text{-}K^+$ ATPase, for restoring the membrane ionic balance after each action potential (Horisberger et al., 1991).

The relative concentrations of adenine nucleotides in the cell (ATP, ADP, AMP), are considered as important regulators of energy metabolism (Newsholme and Leech, 1983; Stryer, 1988).

The ATP-ADP system couples the energy releasing processes of the cell with the contractile process, in such a way that the latter process is totally dependent upon the former (Newsholme, 1988). It has been suggested that energy in the cell is used in a pulsed or fluctuating manner, synchronised with muscle contractions (Wallimann et al., 1992). As a result, transient increases in free ADP and Pi, and decreases in ATP may occur in the vicinity of the ATPases (Ruff and Weismann, 1991; Kingsley et al., 1991; Korge and Campbell, 1994). Large transient changes in the ATP/ADP concentration ratio, are avoided by very rapid and precise mechanisms in
the cell. When the free ADP during contraction increases, the adenylate kinase reaction is activated in order to keep the ATP/ADP ratio high:

$$2\text{ADP} \xrightarrow{\text{adenylate kinase}} \text{ATP} + \text{AMP}$$

The size of the total adenine nucleotide pool (TAN = ATP + ADP + AMP) decreases during short term-high intensity exercise (Sahlin et al., 1978b; Sewell et al., 1991; Stathis et al., 1994). The decrease in ATP concentration after short-term maximal exercise (30 s) is between 30 and 45% (see Table 2.1). At the same time, the increased content of ADP and AMP cannot account for the decrease in [ATP].

2.2.1.1. AMP deamination

This depletion of TAN occurs in contracting muscle when the rate of ATP hydrolysis is excessive, relative to the ability of the cell for ADP rephosphorylation (Terjung et al., 1986; Tullson and Terjung, 1991). Due to the fact that most of the ADP is bound to proteins, a small change in ATP would result in a large relative change in free ADP (ADP\(_f\)) and free AMP (AMP\(_f\)) [e.g. calculated ADP\(_f\) at rest =0.06 → fatigue = 0.3-0.6 mmol·kg dry muscle\(^{-1}\); Foley et al., 1991; Quistcrff. et al., 1992; Meyer and Foley, 1994]. The enzyme AMP deaminase, catalyses the reaction:

$$\text{AMP} + \text{H}_2\text{O} + \text{H}^+ \xrightarrow{\text{AMP deaminase}} \text{IMP} + \text{NH}_4^+$$

where IMP = inosine monophosphate

The function of the reaction is to adjust the adenylate kinase equilibrium, promoting continued formation of ATP from ADP, and therefore keeping the ATP/ADP ratio high (Sahlin and Katz, 1988). It can be calculated that if IMP formation is inhibited, then the increases in ADP\(_f\) and AMP\(_f\) resulting from a decrease of ≈50% in [ATP] would be ≈90-fold and 13,500-fold, compared with ≈8-fold and ≈70-fold when AMP deamination does occur (Dudley and Terjung, 1985; Tullson and Terjung, 1991).

AMP deamination is dependent on the concentration of its substrate AMP\(_f\). A variety of energetically important metabolites have been shown to exert allosteric control on AMP deaminase. These include ADP\(_f\), Pi and H\(^+\) (pH optimum of reaction: 6.2), whose concentration reflects the energetic status of the muscle (Lowestein, 1972; Dudley and Terjung, 1985).
However, acidosis is not a compulsory factor, since measurements in patients with glycolytic enzyme deficiencies (who do not produce lactate) show an increased flux through myokinase and AMP deaminase (Lewis and Haller, 1986; Sahlin et al., 1990). The activity of AMP deaminase has been found to differ between fibre types. Adenine nucleotide degradation occurs readily in fast-twitch, but less in slow-twitch muscle fibres during intense exercise (Meyer and Terjung, 1979; Meyer et al., 1980; Dudley and Terjung, 1985). Studies in humans have shown that IMP accumulation is seen in both fibre types (fast and slow twitch), but the IMP content of the muscle at fatigue is about twice as high in the fast compared with the slow twitch fibres (Sahlin et al., 1989). Furthermore, AMP deaminase activity has been found to be highly correlated with the percentage of fast twitch fibres (Katz et al., 1986b) and phosphofructokinase (PFK) activity (Norman et al., 1994).

2.2.1.2. Ammonia production during high intensity exercise

The irreversible reaction of AMP deamination results in production of ammonia in amounts stoichiometric with IMP (Terjung and Tullson, 1992). Although IMP remains within the cell, ammonia diffuses into the blood (Graham et al., 1993) where it can be easily measured. If exercise is of short duration/high intensity, almost all ammonia production can be attributed to IMP production (Graham et al., 1990, 1993; Terjung and Tullson, 1992). Harris et al. (1991) has found a significant correlation between plasma ammonia and the decline in muscle ATP, and suggested that ATP loss may be evaluated from plasma samples.

Although IMP remains within the cell where it is reaminated, a part of it is further degraded to inosine and then to hypoxanthine which escapes into the circulation (see Fig. 2.2). Both hypoxanthine and uric acid (which is mainly produced from hypoxanthine taken up by the liver, Hellsten, 1994), have been used as indicators of muscle adenine nucleotide loss following high intensity exercise (Hellsten-Westing et al., 1991; Balsom et al., 1992a, 1992b).

A relationship between blood lactate (or pH) and plasma ammonia has been reported in many studies (Babij et al., 1983; Itoh and Ohkuwa, 1991; Nazar et al., 1992). However, this relationship is probably not reflecting cause and effect, but rather the coincidence in the accelerated rates of glycolysis and AMP deamination with intense short-term exercise (Terjung and Tullson, 1992). Ammonia production can be independent of
Fig. 2.2. The purine nucleotide cycle (PNC) and the purine degradation pathway. Deamination of AMP occurs when there is an increase in free AMP from reaction (1), catalysed by adenylate kinase. Numbers indicate the following enzymes: (2) AMP deaminase; (3) adenylosuccinate synthetase; (4) adenylosuccinate lyase; (5) 5'-nucleotidase; (6) adenosine deaminase; (7) purine nucleoside phosphorylase; (8) xanthine dehydrogenase. GTP and GDP, guanosine tri-and di-phosphate; R-1-P, ribose-1-phosphate; NAD and NADH, nicotinamide adenine dinucleotide in the oxidised and reduced form, respectively.
CHAPTER II: Review of Literature

![Diagram of ATP regeneration in muscle]

**Fig. 2.3. Sources of ATP regeneration in muscle.**

Lactate metabolism, and this has been demonstrated both in healthy subjects (Graham *et al.*, 1987), and patients with glycolytic enzyme deficiencies (Sahlin *et al.*, 1990).

### 2.2.2. ATP resynthesis from phosphocreatine degradation

From all the processes in the cell that are used to regenerate ATP (see Fig. 2.3), the creatine kinase reaction is the most powerful (Sahlin, 1986a):

\[
\text{PCr} + \text{ADP} + H^+ \rightleftharpoons \text{creatine kinase} \rightarrow \text{ATP} + \text{Cr}
\]

where \(\text{Cr} = \text{creatine}\)

The muscle content of phosphocreatine (PCr) is 3-4 times higher than that of ATP (see Table 2.1). The activity of creatine kinase is higher than that of ATPase, which means that significant decreases in [ATP] are seen only when PCr is broken down to 60% of the resting value or more (Hultman
et al., 1987). During a 30 s bout of maximal exercise, PCr falls by 60-70% compared to the resting value (see Table 2.1), and can drop to near zero levels during repeated bouts of sprint exercise (McCartney et al., 1986).

The creatine kinase reaction functions to buffer ATP to ADP ratio, and has been considered to act as a stabiliser of intracellular adenylate gradients. It functions as a low-threshold ADP sensor (low $K_m$ of CK for ADP) and plays a critical role in preventing a build-up of ADP, especially during transient periods of high energy utilisation (Wallimann et al., 1992). An increase in free ADP may impair the function of the ATPases by product inhibition (Cooke and Pate, 1990). Furthermore, by maintaining the ATP/ADP ratio high in the vicinity of an ATPase, creatine kinase increases the thermodynamic efficiency ('free energy') of ATP hydrolysis:

$$\Delta G = \Delta G^0 + RT \ln \frac{[ADP][Pi]}{[ATP]}$$

where:
- $\Delta G$ = change in Gibbs' free energy
- $\Delta G^0$ = standard free energy of ATP hydrolysis (-7.3 Kcal·mol$^{-1}$)
- $R$ = gas constant
- $T$ = absolute temperature

The same regulatory and thermodynamic aspects may also hold true for CK at the energy-producing side, where the enzyme is coupled to the ATP-generating systems (e.g. oxidative phosphorylation). In this case CK would be minimizing the free energy required for ATP synthesis.

An important feature of the CK/PCr system is that creatine kinase is expressed as four tissue-specific subunits: two 'cytosolic' forms, M-CK and B-CK (M=muscle; B=brain), and two mitochondrial Mi-CK isoforms. These form three 'cytosolic' and two mitochondrial isoenzymes (Wallimann et al., 1992). The 'cytosolic' CK isoenzymes are functionally coupled with the ATP requiring sites, by being located very close to the 3 different ATPases (Baskin and Deamer, 1970; Ventura-Clapier et al., 1987; Rossi et al., 1990). The mitochondrial isoenzymes have been suggested to play a special role. Bessman and Geiger (1981) and more recently Bessman and Savabi (1990), have proposed that PCr and creatine (Cr) function as a 'shuttle' for the transport of high-energy phosphates between the mitochondrial and myofibrillar CK isoenzymes (Fig. 2.4). Tracer studies have shown that mitochondrially generated ATP has 'preferential' access to the reactive site.
of mitochondrial CK (Erickson-Viitanen et al. 1982). Similarly, a significant fraction of cytoplasmic CK is bound to the M-line of myofibrills, and PCr is reported to be a preferred substrate for myofibrillar phosphate liberation compared with externally supplied ATP itself (Turner et al., 1973; Ventura-Clapier et al., 1987).

The participation of hydrogen ions (H+) in the creatine kinase reaction has two important physiological consequences (Sahlin, 1978):

(a) Breakdown of PCr will absorb H+. This will increase the intracellular pH during the first seconds of maximal contraction. It is thought that this alkalinization will facilitate activation of phosphofructokinase (PFK) and phosphorylase system ('key' enzymes) and accelerate glycolysis.

(b) It has been shown that the log of the apparent equilibrium constant of the creatine kinase reaction and muscle pH are negatively correlated (Sahlin et al., 1975; Harris et al., 1977). Furthermore, a curvilinear relationship between PCr and lactate content in the muscle has been demonstrated, and this relationship was independent of duration,

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Fig. 2.4. Schematic representation of the PCr shuttle, showing the traffic of energy between two isoenzymes of creatine kinase, one bound to the mitochondria (CKmit) and one to the myofibrills (CKmuscle). Adapted from Bessman and Geiger (1981).
CHAPTER II: Review of Literature

Table 2.1. Muscle metabolite concentrations at rest, and after maximal sprint exercise.

<table>
<thead>
<tr>
<th>References</th>
<th>n</th>
<th>Duration</th>
<th>ATP</th>
<th>%ΔATP</th>
<th>PCr</th>
<th>%ΔPCr</th>
<th>Gly</th>
<th>%ΔGly</th>
<th>La post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boobis et al. (1982)#</td>
<td>4M</td>
<td>30 s</td>
<td>24.4</td>
<td>44</td>
<td>84.3</td>
<td>66</td>
<td>261</td>
<td>30</td>
<td>89</td>
</tr>
<tr>
<td>Jacobs et al. (1982)#</td>
<td>9F</td>
<td>30 s</td>
<td>20.9</td>
<td>34</td>
<td>62.7</td>
<td>60</td>
<td>360</td>
<td>23</td>
<td>61</td>
</tr>
<tr>
<td>Boobis et al. (1983)#</td>
<td>7M</td>
<td>30 s</td>
<td>21.2</td>
<td>43</td>
<td>94.4</td>
<td>65</td>
<td>266</td>
<td>21</td>
<td>98</td>
</tr>
<tr>
<td>Jacobs et al. (1983)#</td>
<td>15M</td>
<td>30 s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7F</td>
<td>30 s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Withers et al. (1991)†</td>
<td>5M</td>
<td>30 s</td>
<td>18.5</td>
<td>36</td>
<td>58.8</td>
<td>67</td>
<td>525</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>Stathis et al. (1994)‡</td>
<td>4M,2F</td>
<td>30 s</td>
<td>22.8</td>
<td>40</td>
<td>85.4</td>
<td>68</td>
<td>-</td>
<td>-</td>
<td>92</td>
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<tr>
<td>Cheetham et al. (1986)+</td>
<td>8F</td>
<td>30 s</td>
<td>28.2</td>
<td>37</td>
<td>87.7</td>
<td>64</td>
<td>281</td>
<td>25</td>
<td>78</td>
</tr>
<tr>
<td>Nevill et al. (1989)+</td>
<td>4M,4F</td>
<td>30 s</td>
<td>26.7</td>
<td>28</td>
<td>84.0</td>
<td>67</td>
<td>317</td>
<td>32</td>
<td>86</td>
</tr>
<tr>
<td>Jones et al. (1985)*§</td>
<td>60 revs.min⁻¹</td>
<td>5M</td>
<td>30 s</td>
<td>21.3</td>
<td>37</td>
<td>70.5</td>
<td>60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>140 revs.min⁻¹</td>
<td>5M</td>
<td>30 s</td>
<td>19.1</td>
<td>0</td>
<td>64.8</td>
<td>34</td>
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<td>-</td>
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<tr>
<td>McCartney et al. (1986)*</td>
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<td>30 s</td>
<td>22.6</td>
<td>40</td>
<td>62.0</td>
<td>70</td>
<td>373</td>
<td>21</td>
<td>126</td>
</tr>
<tr>
<td>Spriet et al. (1989)*</td>
<td>sprint 2</td>
<td>7M,1F</td>
<td>30 s</td>
<td>17.7</td>
<td>10</td>
<td>39.0</td>
<td>49</td>
<td>319</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>sprint 3</td>
<td>7M,1F</td>
<td>30 s</td>
<td>17.3</td>
<td>11</td>
<td>36.9</td>
<td>55</td>
<td>287</td>
<td>5</td>
</tr>
<tr>
<td>Boobis et al. (1982)#</td>
<td>4M</td>
<td>6 s</td>
<td>24.4</td>
<td>9</td>
<td>84.3</td>
<td>35</td>
<td>261</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>Gaitanos et al. (1993)#</td>
<td>8M</td>
<td>6 s</td>
<td>24.0</td>
<td>13</td>
<td>76.5</td>
<td>57</td>
<td>317</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td>Jacobs et al. (1983)#</td>
<td>15M</td>
<td>10 s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7F</td>
<td>10 s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jones et al. (1985)*§</td>
<td>2M</td>
<td>10 s</td>
<td>14.7§</td>
<td>-</td>
<td>19.4§</td>
<td>-</td>
<td>-</td>
<td>66§</td>
<td></td>
</tr>
</tbody>
</table>

Gly, muscle glycogen; La⁺, muscle lactate; PCr, phosphocreatine. #, friction-loaded cycle ergometer; M = males; F = females; †, non-motorised treadmill; *, isokinetic cycle ergometer; ‡, air braked cycle ergometer. § means for sprints at 60 and 140 revs·min⁻¹, only post sprint values reported; Values are in mmol·kg dry muscle⁻¹ or are expressed as a percentage of the resting value.

intensity and type of exercise performed (Harris et al., 1977). Therefore, it follows that a decrease in intracellular pH will affect PCr concentration by displacing the creatine kinase equilibrium towards PCr breakdown. PCr can supply energy at very high rates during the first few seconds of intense exercise. However, the limited muscle content of PCr can not support these rapid rates for a long period. Therefore, the glycolytic pathway is activated almost immediately after the start of intense contractions (Hultman and Sjoholm, 1983; Shoubridge and Radda, 1987). The link between phosphocreatine breakdown and glycogen utilisation in the muscle, is thought to be the inorganic phosphate (Pi) liberated during ATP-PCr degradation (Chasiotis et al., 1982a; Chasiotis, 1988). The
mechanisms by which glycogenolysis† and anaerobic glycolysis‡ are activated by Pi will be discussed in section 2.2.3.1.

2.2.2.1. Effects of changes in muscle PCr content on metabolism and exercise performance.

The importance of the CK/PCr system for muscle metabolism and performance can be demonstrated by experimentally manipulating the PCr content of the muscle. Studies have involved manipulations in both directions: (i) creatine–PCr depletion and (ii) creatine–PCr elevation.

Creatine biosynthesis occurs in tissues other than the muscle (liver, kidney, pancreas) and the creatine content of the body can be affected by diet (Crim et al., 1976). Since muscle PCr concentrations are maintained in part by entry of newly synthesised creatine, an increased dietary intake of creatine can increase muscle PCr stores (Harris et al., 1992). Conversely, inhibition of creatine entry in the muscle (e.g. by oral administration of creatine analogues) would result in a depletion of PCr stores (Fitch 1968, 1974; Shoubridge et al., 1985; Meyer et al., 1986).

(i) Creatine depletion

Creatine depletion studies have been performed in rats. Feeding rats with the creatine analogue β-guanidinopropionic acid (GPA) for 6-10 weeks lead to a ~90% depletion of PCr and a 20-50% decrease of CK and glycolytic (PFK) activities while oxidative capacity and the proportion of slow twitch fibres were increased (Fitch, 1974; Shoubridge et al., 1985; Meyer et al., 1986; Moreland et al., 1989). As a result of these metabolic changes, muscle fatigued faster during electrical stimulation (Meyer et al., 1986; Shoubridge and Radda, 1987), and it has been suggested that lack of phosphate (Pi) liberation caused by reduced PCr breakdown may be related to the observed decreased activation of glycogenolysis/glycolysis (Meyer et al., 1986).

† For the purposes of this review, glycogenolysis is defined as an estimate of glycogen breakdown, reflecting the activity of the flux-generating enzyme glycogen phosphorylase. This enzyme catalyses the phosphorolysis (splitting by inorganic phosphate) of terminal glucose units from the non-reducing end of glycogen to yield glucose-1-phosphate.

‡ Anaerobic glycolysis is defined as an estimate of the degradation of glucose-6-phosphate to pyruvate and lactate, reflecting the activity of the rate-limiting enzyme phosphofructokinase (PFK).
(ii) Creatine supplementation

Harris et al. (1992) has demonstrated that oral creatine supplementation (20-30 g·day⁻¹) for as little as 5 days increased muscle total creatine (PCr+creatine) by ~20%, of which 20-30% was in the form of PCr. More recently, Greenhaff et al. (1994c) reported a similar result, but also showed that PCr resynthesis during the 2nd min of the recovery period after electrical stimulation (32 s at 50 Hz) was enhanced by ~35% following creatine supplementation. An interesting finding, which was also seen in the study of Harris et al. (1992) was that there were subjects who did not 'respond' to the supplementation. In both studies these subjects had relatively high initial total creatine, which would suggest that the initial level itself may set the limit for further increases. A very important aspect of creatine supplementation is the improvement of performance during repeated bouts of maximal exercise, following a short period of creatine ingestion (Balsom et al., 1993; Greenhaff et al., 1993; Earnest et al., 1994; Birch et al., 1994). Despite the increased power output after creatine supplementation in these studies, plasma ammonia or hypoxanthine accumulation were decreased, implying a more efficient ADP rephosphorylation. Indeed, Greenhaff et al. (1994b) has shown that although work output was increased when subjects performed a second 30 s bout of maximal isokinetic cycling after creatine ingestion, ATP loss was reduced by 50%. In contrast to the positive effects on short duration-high intensity exercise, there is no improvement in endurance performance after creatine supplementation (Balsom et al., 1993b).

The exact mechanism which results in improvements during high intensity exercise performance is unknown. An interesting question is whether or not the increase in total creatine is accompanied by an increase in total phosphate (as Pi is part of muscle buffering capacity, see section 2.3.3.). Another topic which deserves further investigation is whether the effects of the increased PCr are purely due to the extra energy, or due to a more efficient removal of end products of the ATPases by the CK/PCr system.

2.2.3. Glycolytic energy supply during high intensity exercise

In comparison with the high energy phosphates (ATP, PCr), the content of glycogen in the muscle is larger (see Table 2.1). Muscle glycogen and glucose can be utilised in the glycolytic pathway and rephosphorylate ADP
at high rates. The intracellular content of free glucose and endogenous hexose phosphates is very low, and the penetration/transport across the cell membrane is a slow process, compared with the rate of local glycogen degradation (1:100) (Hultman and Sjoholm, 1983). Therefore, muscle glycogen is the main substrate for anaerobic glycolysis during high intensity exercise of short duration.

All the reactions of anaerobic glycolysis occur in the cytosolic compartment of the cell, where it is generally considered that the enzymes involved are in free solution, and that the transfer of intermediates from one enzyme to the next occurs by diffusion (Newsholme and Leech, 1983; see Fig. 2.5).

The process of glycogen-to-lactate degradation (glycogenolysis+anaerobic glycolysis) results in the net formation of 3 moles of ATP per glucosyl unit, in comparison to 39 moles ATP per glucosyl unit, when glycogen is degraded aerobically (Stegemann, 1981). The generation of ATP occurs by substrate phosphorylation, at the phosphoglycerate kinase and pyruvate kinase steps (Martin et al., 1983).

A summary of the reactions involved in glycogen-to-lactate degradation:

\[
\text{Glycogen}_{(n)} + 3\text{ADP} + 3\text{Pi} \rightarrow \text{Glycogen}_{(n-1)} + 3\text{ATP} + 2\text{La}^- + 2\text{H}^+
\]

The breakdown of glycogen is initiated by the action of the enzyme phosphorylase, which is specific for the removal of glucose units (1,4-glucosyl residues) from the outermost chains of the glycogen molecule, until approximately 4 glucose residues remain before a -1,6-branch point. The remaining glucose units are made available to phosphorylase through the activity of debranching enzyme. The first activity of the debranching enzyme (glucosyl transferase) is to transfer three out of the remaining four glucose units to another chain for further degradation by phosphorylase, whereas the second activity of the enzyme (amylo-1,6-glucosidase) breaks the α-1,6 branch point linkage and releases the last unit as free glucose (Martin et al., 1983). It has been estimated that ≈7% of the glucose residues in glycogen are released as free glucose and not glucose-1-phosphate. This may explain the increase of free muscle glucose (6-7% of glycogen degraded) during short duration electrical stimulation of the quadriceps with the circulation occluded (Spriet et al., 1987b; Soderlund and Hultman, 1991).
Fig. 2.5. The metabolic pathway of glycolysis (in Martin et al., 1983)
Many studies have shown that muscle glycogen is the dominant fuel for ATP production during short-term, intense exercise (Jacobs et al., 1982, 1983; Cheetham et al., 1986; McCartney et al., 1986; Spriet et al., 1989). The percent contribution of anaerobic glycolysis to total anaerobic ATP turnover during a 30 s sprint is 60-70% (Cheetham et al., 1986; Boobis et al., 1982). Considerable evidence exists to show that glycolysis contributes to ATP production, from the first few seconds of high intensity exercise. Hultman and Sjoholm (1983) reported that after 1.26 s and 2.56 s of electrical stimulation of the quadriceps femoris muscle at 50 Hz, 20% and 50% of the total ATP turnover, respectively, was derived from degradation of glycogen to lactate (blood flow was occluded). Moreover, short-term sprint cycling exercise (6-10 s), results in a relatively high muscle lactate accumulation, which is between 25 and 60% of that achieved during a 30 s bout (Boobis et al., 1982; Jacobs et al., 1983; Jones et al., 1985; Gaitanos et al., 1993). It seems, therefore, that both PCr and glycogen degradation achieve their maximum rates at the beginning of sprint exercise, but these rates decrease as the exercise continues.

The glycogen stores in skeletal muscle are reduced only by a small percentage (20-30%) after a 30 s sprint bout (see Table 2.1). Although it is unlikely that glycogen availability can limit exercise performance during short term sprinting, analysis of metabolites in single fibres following electrical stimulation or short lasting very intense exercise, has shown a ≈2-fold greater decrease in glycogen in the fast, compared with the slow twitch fibres (Greenhaff et al., 1991, 1993; Soderlund et al., 1992; Vollestad et al., 1992; ). However, the glycogen content itself in the fast twitch fibres was not very low at the end of the exercise. An attractive consideration is that glycogen may become depleted in some parts of the fibre. This suggestion is based on the findings of Hintz et al. (1984) who measured enzymes and metabolites at different segments along the length of single fibres and observed a heterogeneous distribution of phosphorylase and glucose-6-phosphate concentration after stimulation.

2.2.3.1. Metabolic regulation of glycogen metabolism

Phosphorylase catalyses the flux-generating step for glycogen conversion to lactate in the muscle, and therefore is central to the regulation of glycolysis. The control of phosphorylase activity is complex and involves at least three different control mechanisms, acting in concert (Newsholme and Leech, 1983). These include:
• Hormonal mechanisms,
• Ca\(^{2+}\) 'stimulation' of phosphorylase
• control by "external regulators" (altered muscle metabolites).

These mechanisms control the transformation of the inactive \(b\) form of phosphorylase, to the active \(a\) form. The fraction of phosphorylase \(a\) in resting muscle is about 22-30% of total phosphorylase, but this increases rapidly to 60-90% during muscle activity (Chasiotis et al., 1982a).

![Diagram](image)

*Fig. 2.6. Regulation of glycogen phosphorylase activity in muscle. Activation of phosphorylase kinase \(b\) requires a higher concentration of Ca\(^{2+}\) than does the activation of phosphorylase kinase \(a\) (in Newsholme and Leech 1983).*

It has been known for many years that adrenaline stimulates glycogen breakdown in the muscle, via a rise in the concentration of cyclic AMP (cAMP; Newsholme and Leech, 1983; Martin et al., 1983). Adrenaline increases the activity of adenylate cyclase, which catalyses the formation of cAMP from ATP. Increase in cAMP activates the enzyme protein kinase, which phosphorylates phosphorylase kinase, and that finally leads to an increase of the active \(a\) form of phosphorylase (see Fig. 2.6; Chasiotis et al., 1982a; Chasiotis et al., 1983a). The fact that several enzymes are involved in the whole sequence for the conversion of phosphorylase \((b)\) to phosphorylase \((a)\), provides a greater sensitivity of the system (Newsholme and Leech, 1983).

Plasma adrenaline levels during short-term, high intensity exercise (30 s), have been shown to increase by \(\approx 4-10\) fold (MacDonald et al., 1983; Cheetham et al., 1986; Brooks et al., 1988, 1990). The effect of adrenaline on glycogenolysis during contractions has been investigated by adrenaline...
infusion (or in the case of animal studies, muscle perfusion) before and during electrical stimulation. The results of these studies showed that during intense exercise adrenaline enhanced glycogenolysis in slow twitch fibres, whereas there was no effect of adrenaline on the fast twitch fibres (Richter et al., 1982; Greenhaff et al., 1991; Chesley et al., 1994).

An important finding of the adrenaline infusion studies was that despite an almost total transformation of phosphorylase to the active $a$ form following infusion, glycogenolysis was extremely low when muscle was at rest (Chasiotis et al., 1982a, 1983a; Ren and Hultman, 1990). This led to the conclusion that other factors are more important for glycogen breakdown regulation.

An increase in $[\text{Pi}]$, which is one of the substrates of phosphorylase, was considered to be a major determinant of glycolysis (Chasiotis et al., 1982a, 1983a, 1983b). This Pi comes from ATP-PCr splitting, and is a main link in the "chain" of metabolic responses to exercise. Chasiotis et al., (1982a) argued that the dianion form of Pi ($\text{HPO}_4^{2-}$) is the "real" substrate of phosphorylase. Therefore, a drop in muscle pH which increases $\text{Pi}$ in its acidic form ($\text{H}_2\text{PO}_4^-$) may affect glycogenolysis. Despite the important role of Pi for the regulation of glycogenolysis (Ren et al., 1988), studies using occlusion of the circulation after intense muscle contractions have provided evidence suggesting that elevated Pi in combination with extensive phosphorylase transformation are not enough to elicit a high glycogenolytic rate (Ren and Hultman, 1989).

Allosteric modulation of phosphorylase by AMP has been proposed to be an important regulator of muscle glycogenolysis. Chasiotis (1983) showed that the $K_m$ of phosphorylase $a$ for Pi was 27 mmol·l$^{-1}$ in the absence of AMP, but was decreased to 7 mmol·l$^{-1}$, when AMP was added. Similarly, Ren and Hultman (1990) showed that a small amount of AMP could increase the affinity of phosphorylase for Pi, and thus phosphorylase $a$ activity. In the same study, Ren and Hultman (1990) observed a high correlation between the rate of ATP utilisation and the rate of glycogenolysis. They concluded that events related to the contraction intensity (such as transient increases in AMP) may regulate the glycogenolytic activity of phosphorylase $a$. However, the importance of an increase in AMP was disputed in a recent $^{31}$P-MRS study by Quistorff et al. (1992). They used ischaemia to maintain the high level of free ADP, free AMP and Pi during the rest period following isometric calf muscle
contraction. They found that glycogenolysis and glycolysis were arrested despite the high levels of activators, and argued that some other factor related to contraction 'switches on and off' the glycogenolytic and glycolytic processes.

The link between muscle contraction and activation of glycolysis is Ca\(^{2+}\). Nervous stimulation of a muscle causes a release of Ca\(^{2+}\) from the sarcoplasmic reticulum, which initiates contraction by stimulating myofibrillar ATPase. At the same time, Ca\(^{2+}\) also stimulates the conversion of phosphorylase \(b\) to the \(a\) form (Brostrom et al., 1971; Drummond et al., 1969; Newsholme and Leech, 1983). A significant point is that the concentration of Ca\(^{2+}\) required to activate phosphorylase kinase is considerably lower if the kinase is phosphorylated (by cyclic AMP activation of protein kinase; see Fig. 2.6).

Thus, it seems that glycogenolysis is controlled in such a way that it 'switches on' only when contraction occurs. However, all the modulators of glycogenolysis mentioned above are important for the regulation of glycogenolytic rate. For example, the partial reversal of phosphorylase back to the \(b\) form and decrease in glycogenolysis which has been observed in electrically stimulated rat muscle (Conlee et al., 1979) may have been due to the lack of hormonal (adrenaline) activation. The role of adrenaline as an anticipatory hormone is to convert phosphorylase kinase to the \(a\) form which is more sensitive to Ca\(^{2+}\) (see Fig. 2.6), thereby increasing the responsiveness of the "system" to muscle contraction (Newsholme and Leech, 1983).

Further in the glycolytic pathway, the non-equilibrium reactions of phosphofructokinase (PFK) and pyruvate kinase are regulated by 'external factors' (see Fig. 2.7). The most important regulation is thought to occur by the PFK reaction.

During short-term sprint exercise, the glycolytic intermediates above PFK increase considerably (≈ 13fold), suggesting a rate limiting step (Cheetham et al., 1986; Boobis et al., 1987). As judged by the marked accumulation of hexose monophosphates, degradation of glycogen generally seems to occur at a rate "in excess" of the glycolytic rate in this type of exercise. Accumulation of hexose monophosphates removes part of the Pi liberated as a result of PCr utilisation, thereby indirectly controlling glycogenolysis (Chasiotis and Hultman, 1983).
Although PFK has been shown to be extremely sensitive to pH changes at the physiological pH range (Trivedi and Danforth, 1966), its activity during exercise is determined by the net effect of the changes in all inhibitors and de-inhibitors (activators) which regulate the enzyme (see Fig. 2.7; Dobson et al., 1986; Spriet et al., 1987b; Hultman and Harris, 1988). Electrical stimulation studies in humans have shown that despite large decreases in muscle pH, substantial glycolytic (PFK) activity is preserved due to decreases in ATP and PCr and increases in ADP, AMP, Pi and fructose-6-phosphates (Spriet et al., 1987b). Physiological concentrations of ATP exert an allosteric inhibition on PFK. This inhibition is potentiated by high ATP, PCr, H+ and citrate concentrations. De-inhibitors of the ATP inhibition include AMP, ADP, Pi, fructose-6-phosphate (the reaction substrate), fructose 1,6-bisphosphate, fructose 2,6-bisphosphate, glucose 1,6-bisphosphate and ammonia (Newsholme and Leech, 1983; Lowenstein, 1972; Dobson et al., 1986). However, measurements of muscle ammonia...
during and after isometric contraction to fatigue (~1 min) suggested that ammonia did not have any important influence on glycolysis (Katz et al., 1986b).

An interesting concept regarding the control of glycolytic rate, especially at the PFK step, is that of the "substrate cycles" or "futile cycles" (Newsholme and Leech, 1983; Newsholme, 1988). Since only small changes of the enzyme's metabolic regulators occur during contraction, the system must be very sensitive. It is suggested that this is achieved by co-operativity and the operation of a "substrate cycle" between fructose-6-phosphate and fructose bisphosphate, catalysed by the simultaneous activities of PFK (forward) and fructose bisphosphatase (reverse; see Fig. 2.8). Such cycles provide high sensitivity only if the rate of cycling, compared with the net flux through the pathway, is high.

Newsholme (1988) described the mechanism by which this substrate cycle works. In anticipation of a high intensity activity (e.g. a race), the increased adrenaline level causes a high rate of "cycling", but since no muscular work is done the glycolytic flux is still low. The initiation of the race increases the metabolic stimulators of PFK, and only a small increase in PFK activity (e.g 25 fold) coupled with a minor decrease in fructose bisphosphatase, causes a 1000 fold increase in flux (see Fig. 2.8).

2.2.4. Oxidative contribution to ATP resynthesis during high intensity exercise

Numerous studies have shown that the contribution of aerobic metabolism to energy release increases with the duration of exercise (Medbo and Tabata, 1989).

The mean power output during the "Wingate test" (30 s all out sprint cycling), was assumed to represent the "anaerobic capacity", i.e. the maximum amount of anaerobic energy release during exercise (Bar-Or, 1978). Despite the high anaerobic contribution to energy supply during a 30 s sprint, the amount of energy that can be provided by aerobic metabolism can be significant (Vandewalle et al., 1987a).

Several studies have attempted to calculate the percent contribution of aerobic and anaerobic metabolism during a 30 s all out sprint. However these calculations were based on several assumptions, and the results must be considered with caution. The lack of information on the actual
mechanical efficiency of supramaximal exercise (>\(\text{VO}_2\text{max}\)), is probably the major source of error (Bedu et al., 1991).

Nevertheless, values between 18% and 40% of aerobic contribution have been reported for 30 s high intensity cycling exercise (Kavanagh and Jacobs, 1988; Serressse et al., 1988; Medbo and Tabata, 1989). The value of 40% for aerobic contribution during a 30 s exercise bout reported by Medbo and Tabata (1989) is probably an overestimation for sprinting for the following reasons: a) it was calculated using the method of oxygen deficit, which assumes linearity between \(\text{O}_2\) uptake and power output at supramaximal (>\(\text{VO}_2\text{max}\)) intensities and b) the exercise bout was performed at a constant, and relatively slow pedalling frequency (90 revs.min\(^{-1}\)).

Values for aerobic contribution obtained from calculations based on \(\text{VO}_2\) during the sprint, mechanical efficiency and external work, can vary greatly depending on the assumptions made. Furthermore, oxygen stored in venous blood and in the muscle is not taken into account. The available oxygen from these stores corresponds to \(\approx 12\) mmol ATP·kg dry muscle\(^{-1}\), as estimated by Harris et al. (1975).

![POWER OUTPUT (W·kg\(^{-1}\))](image)

**Fig. 2.9.** Power output (sprint) and \(\text{VO}_2\) kinetics (aerobic), during cycle ergometer sprinting. \(P_{\text{max}}\) corresponds to power output at \(\text{VO}_2\text{max}\). Area 3 (dotted): aerobic contribution to a 30 s sprint test. Maximum anaerobic capacity: sum of areas 1,2,4,5 (adapted from Vandewalle et al., 1987b).
The significant contribution of aerobic metabolism to a 30 s all out cycle ergometer sprint, has been demonstrated by breathing hypoxic gas mixtures (12% O₂) while performing the test (Kavanagh et al., 1986). A significant drop of mean power output in the hypoxic condition was assumed to reflect the partial dependence on aerobic metabolism. Similar results were obtained when the test was performed at a high altitude (Bedu et al., 1991). Further (indirect) evidence for the importance of aerobic contribution during repeated bouts of high intensity exercise has been provided by administering the red blood cell producing stimulant, erythropoietin for 6 weeks (Balsom et al., 1994). A decreased blood lactate and plasma hypoxanthine accumulation were observed when the test (15 x 6 s, separated by 24 s rest) was repeated after erythropoietin administration, and this was assumed to reflect an increased contribution of aerobic metabolism.

It has been suggested that subjects with higher VO₂max values, have a larger relative aerobic contribution during a 30 s all-out cycle sprint (Kavanagh and Jacobs, 1988). In that study, the subjects attained 85 ± 17% of their VO₂max during the last five seconds of the sprint, demonstrating very fast VO₂ kinetics. A diagrammatic representation of the VO₂ kinetics and power output during a 30 s sprint cycling test, is shown in Fig. 2.9.

Fig. 2.10. Regulation of pyruvate dehydrogenase. Under the influence of kinase, ATP phosphorylates pyruvate dehydrogenase a, thereby inactivating it. Removal of the phosphate by the phosphatase, activates pyruvate dehydrogenase (adapted from Newsholme and Leech, 1983).
Although the ATP turnover rates during a 30 s sprint cycling bout are significantly higher than the aerobic processes can attain (≈6-7 vs 2.7 mmol·kg dry muscle$^{-1}$·s$^{-1}$; Cheetham et al., 1986; Sahlin, 1986a), the aerobic contribution may be important for the last part of the 30 s bout, especially during repeated 30 s bouts.

McCartney et al. (1986) have suggested that the relatively high mean power output (≈400 W) sustained during the last two 30 s bouts (4 x 30 s isokinetic cycling sprints, separated by 4 min), was due to an increased energy release from aerobic metabolism. This was supported by the 500% increase in blood glycerol, which was taken to reflect utilisation of intramuscular triglycerides. A significant aerobic contribution to repeated bouts of high intensity exercise has also been suggested by Spriet et al., (1989) during three 30 s isokinetic cycling sprints separated by 4 minutes of recovery, and by Gaitanos et al. (1993) during ten 6 s sprints interspersed by 30 s.

Spriet et al. (1989) has also proposed that a higher oxygen consumption during repeated sprints (not measured in their study), may be accompanied by an increase in the activity of pyruvate dehydrogenase (PDH), resulting in greater oxidation of pyruvate. It was argued that PDH and phosphorylase may be regulated by the same metabolic changes (e.g. elevated H$^+$), but in the opposite direction (i.e. inhibition of phosphorylase and activation of PDH).

Fig. 2.10 shows the factors regulating pyruvate dehydrogenase. In contrast to glycogen phosphorylase, phosphorylation of PDH leads to inactivation of the enzyme (formation of the $b$ form by action of protein kinase), while dephosphorylation leads to activation (formation of the $a$ form, mediated by phosphatase). The modulators of PDH transformation include the following (Newsholme and Leech, 1983; Constantin-Teodosiu, 1992; Ward et al., 1982):

**POSITIVE MODULATORS:**
- pyruvate and ADP which inhibit the kinase
- calcium both activates phosphatase and inhibits kinase

**INHIBITORS:**
- high ATP/ADP ratio
- high acetyl CoA/coenzyme A ratio
- high NADH/NAD$^+$ ratio

Although it has been argued that increased H$^+$ observed during short-term intense exercise may inhibit PDH transformation (Ward et al., 1982), Constantin-Teodosiu (1992) has observed complete transformation when muscle lactate levels were very high (130 mmol·kg dry muscle$^{-1}$).
Furthermore, Newsholme and Leech (1983), reported that a fall in pH will increase PDH activity, so that pyruvate oxidation and hence proton utilisation will be increased. Data from preliminary studies measuring PDH transformation during repeated 30 s sprints on an isokinetic cycle ergometer, have shown that PDH is almost completely transformed to the active form during sprinting, while the percentage PDH in the active form decreases during the 4 min of recovery (Putman et al., 1994).

2.3. CHANGES IN ACID-BASE BALANCE DURING HIGH INTENSITY EXERCISE

2.3.1. Muscle lactate and pH

The term pH was introduced in 1909 by Sørensen, who defined pH as the negative logarithm of the hydrogen ion activity (in Martin et al., 1983). However, in dilute solutions activity and concentration can be considered to be the same, and thus pH becomes the negative logarithm of the hydrogen ion concentration (see APPENDIX E):

\[ \text{pH} = -\log [H^+] \]

Low pH (acidic) values (below 7.0) correspond to high concentrations of \( H^+ \), and high pH (basic) values (above 7.0) to low concentrations of \( H^+ \) (see APPENDIX E for conversions). The pH of the resting human muscle is \( \approx 7.0 \), and it is decreased by \( \approx 0.5 \) pH units at fatigue\(^\dagger\) (see Table 2.2). The fact that during high intensity intermittent exercise muscle pH decreased to about the same value (\( \approx 6.4 \)) after each bout (Hermansen and Osnes, 1972), has led to the suggestion that this decrease in muscle pH is the limiting factor during maximal exercise of short duration.

Although several metabolic processes in the muscle cell release or consume \( H^+ \), lactic acid is considered to be the main origin of \( H^+ \) accumulation and thus a decrease in pH (Hultman and Sahlin, 1980). A

\( \dagger \) muscle pH measured by the homogenate technique represents the average pH of intra- and extra-cellular compartments (Hultman and Sahlin, 1980). When a pH electrode is inserted in the muscle, it measures pH of the extracellular fluids (Allsop et al., 1990). \( ^{31}\text{P}-\text{MRS} \) (magnetic resonance spectroscopy) gives a measure of the 'true' intracellular pH (Wilson et al., 1988).
close relationship between muscle lactate accumulation and decrease in muscle pH is usually seen after intense exercise (Sahlin et al., 1976; Harris et al., 1989; Spriet et al., 1987b; Mannion et al., 1993). Lactic acid is a strong acid (pK\(^*\) = 3.8) and at physiological pH, is almost completely dissociated to lactate ions (La\(^-\)) and hydrogen ions (H\(^+\)). The H\(^+\) are, therefore, formed in an amount equivalent to lactate. The contribution of lactic acid to the total H\(^+\) production in the muscle has been estimated by Sahlin (1986b) to be > 85%. The same author suggested that most of the produced H\(^+\) will be buffered within the tissue and only a small fraction (<0.001%) will appear as free ions, which nevertheless will cause a decrease in pH.

A way to assess the contribution of changes in muscle metabolites (other than lactate) to the increased muscle acidity is to calculate the 'strong ion difference'. This concept is based on physicochemical interactions between 'independent' and 'dependent' (e.g. [H\(^+\)]) variables within the constraints imposed by the laws of electrical neutrality and conservation of mass. In simple words, the procedure involves solving equations (which will give [H\(^+\)]), after measuring the concentration of the most important 'independent variables' in the muscle (or blood), namely:

(a) 'strong' (fully dissociated) ions\(^*\), i.e. Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Cl\(^-\) and La\(^-\),
(b) the total concentration of weak (partially dissociated) acids, mainly proteins and phosphates, and
(c) PCO\(_2\)

Using this method, Kowalchuk et al. (1988a) calculated that immediately after a 30 s sprint, the changes in muscle lactate almost fully accounted for the decrease in [SID] and therefore pH. However, during recovery (3.5 and 9 min) muscle [SID] remained low due to the decreased muscle [K\(^+\)], despite a significant decrease in [La\(^-\)]. This shows that muscle lactate may not be the only factor influencing [H\(^+\)] in the muscle and blood during and after exercise. An interesting point emerging from similar calculations of [SID] in the blood is that the decrease in blood pH seen immediately after a 30 s sprint, which usually exceeds La\(^-\) accumulation, may be the result of a

\* pK = -logK, where K is the dissociation constant which expresses the tendency to ionize. The pK of an acid group can be defined as the pH at which the protonated and unprotonated species are present at equal concentrations. Note that the stronger acid groups (which are completely dissociated even at low pH), have lower pK values (Martin et al., 1983).

\(\dagger\) Strong ion difference (SID) is the difference between the sum of the strong basic cations and the sum of the strong acid anions: [SID] = [Na\(^+\) + K\(^+\) + Ca\(^{2+}\) + Mg\(^{2+}\)] - [Cl\(^-\) + La\(^-\)]
Table 2.2. Muscle and blood pH at rest and following high intensity exercise in humans.

<table>
<thead>
<tr>
<th>References</th>
<th>EXERCISE type, intensity, duration</th>
<th>MUSCLE pH Pre</th>
<th>Post</th>
<th>BLOOD pH Rest</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hermansen &amp; Osnes (1972)</td>
<td>C/running to exhaustion 100 s</td>
<td>6.92</td>
<td>6.41</td>
<td>7.42</td>
<td>7.11</td>
</tr>
<tr>
<td>Sahlins et al. (1976)</td>
<td>C 60 revs-min⁻¹</td>
<td>7.08</td>
<td>6.60</td>
<td>7.42</td>
<td>7.18</td>
</tr>
<tr>
<td>King et al. (1985)</td>
<td>Isokinetic C, 90 revs-min⁻¹ 45 s</td>
<td>7.15</td>
<td>6.67</td>
<td>7.37</td>
<td>7.13</td>
</tr>
<tr>
<td>Bell &amp; Wenger (1988)</td>
<td>one-legged maximal C 60 s</td>
<td>6.92</td>
<td>6.59</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spriet et al. (1989)</td>
<td>Isokinetic C, 100 revs-min⁻¹ 3x30 s, 4 min rest; sprint 2 30 s</td>
<td>6.71</td>
<td>6.56</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>6.65</td>
<td>6.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannion (1990)</td>
<td>maximal sprint C 44.5 s</td>
<td>7.20</td>
<td>6.73</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Costill et al. (1983)</td>
<td>400m maximal sprint 60.4 s</td>
<td>7.03</td>
<td>6.63</td>
<td>-</td>
<td>7.10</td>
</tr>
<tr>
<td>Nevill et al. (1989)</td>
<td>T sprint running 30 s</td>
<td>7.02</td>
<td>6.80</td>
<td>7.38</td>
<td>7.16</td>
</tr>
<tr>
<td>Allsop et al. (1990) e</td>
<td>T sprint running 30 s</td>
<td>7.17</td>
<td>6.57</td>
<td>7.39</td>
<td>7.04</td>
</tr>
<tr>
<td>Sahlin et al. (1975)</td>
<td>Isometric 68% MVC 25 s</td>
<td>7.09</td>
<td>6.75</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 s</td>
<td>6.56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sahlin &amp; Henriksson (1984)</td>
<td>Isometric 61% MVC 50.6 s</td>
<td>7.12↑</td>
<td>6.61↑</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57.2 s</td>
<td>7.10#</td>
<td>6.80#</td>
<td>-</td>
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<tr>
<td>Spriet et al. (1987b)</td>
<td>I.E.S., 20 Hz 1.6 s contraction/rest</td>
<td>25.6 s</td>
<td>7.00</td>
<td>6.70</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>51.2 s</td>
<td>6.60</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
<td>76.8 s</td>
<td>6.45</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>102.4 s</td>
<td>6.43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannion (1990)</td>
<td>Isometric 60% MVC 59 s</td>
<td>7.17</td>
<td>6.94</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>b) 31P-MRS STUDIES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baker et al. (1992)</td>
<td>Isometric MVC ankle dorsiflexion</td>
<td>120 s</td>
<td>7.07</td>
<td>6.53</td>
<td></td>
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<tr>
<td>Wilson et al. (1988)</td>
<td>Isokinetic wrist flexion 4 min</td>
<td>7.01</td>
<td>6.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McCully et al. (1994)</td>
<td>Fast ankle plantarflexion 64 s</td>
<td>7.06</td>
<td>6.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vandenborne et al. (1991)</td>
<td>Fast ankle plantarflexion 120 s (30% of max single lift)</td>
<td>7.00</td>
<td>6.63 (high pH peak)</td>
<td>6.21 (low pH peak)</td>
<td></td>
</tr>
<tr>
<td>Yoshida &amp; Watari (1993a)</td>
<td>Dynamic knee flexion 4 min</td>
<td>7.00</td>
<td>6.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mizuno et al. (1994)</td>
<td>Wrist and finger flexion 8 min</td>
<td>7.03</td>
<td>6.78 (high pH peak)</td>
<td>6.34 (low pH peak)</td>
<td></td>
</tr>
</tbody>
</table>

C, cycling; T, non-motorised treadmill; I.E.S., intermittent electrical stimulation; MVC, maximal voluntary contraction; t, sedentary subjects; #, trained subjects; e, measured by intramuscular pH electrode. 31P-MRS, magnetic resonance spectroscopy.
large rise in PCO\textsubscript{2} due to the high CO\textsubscript{2} efflux from the muscle to the blood during the initial 30 s of recovery (Kowalchuk et al., 1988a; Brechue and Stainsby, 1994). In this context, muscle perfusion (to remove CO\textsubscript{2} and ions) and the lungs (to eliminate CO\textsubscript{2}) act in concert, and play a major role in the recovery of muscle pH during the first few minutes of recovery after sprint exercise (Kowalchuk et al., 1988b).

2.3.2. Blood lactate and pH

It is well documented that intense short-term exercise results in pronounced changes in blood lactate and pH (Hermansen and Osnes, 1972; Costill et al., 1983; Cheetham et al., 1986; Cheetham, 1987; Nevill et al., 1989). The release of lactate and protons from the muscle to the blood during and after the exercise bout, increases blood [La\textsuperscript{-}] and decreases blood pH. The peak blood lactate concentration after a 30 s all-out sprint, is usually attained 5-6 min into recovery (Cheetham et al., 1986; Jones et al., 1985), and is between 12 and 16 mmol\cdot l\textsuperscript{-1}. Repeated 30 s bouts on an isokinetic cycle ergometer, can increase the blood lactate concentration up to 21-23 mmol\cdot l\textsuperscript{-1}, (McCartney et al 1986; 4 x 30 s separated by 4 min), while the rate of blood lactate accumulation is decreased with every sprint. Similar findings have been reported by Rieu et al. (1988), for four 45 s treadmill runs at \textasciitilde 175\% VO\textsubscript{2max}.

2.3.2.1. Lactate and H\textsuperscript{+} translocation from muscle to blood

During transient periods of rapid change in the transmembrane lactate gradient, membrane characteristics are more likely to be the limiting factor in lactate translocation (Gladden, 1989). Permeation could occur with lactate in ionic form or as an undissociated acid by: (a) passive diffusion, (b) passive mediated transport, and (c) an active mediated process (Hultman and Sahlin 1980).

In the past, investigators assumed that lactate moves rapidly across muscle membranes by simple diffusion. However, the large gradients between muscle and blood or extracellular medium during exercise (Gladden and Yates, 1983), suggest, but do not prove, that there is a membrane hindrance to translocation. If a lactate carrier plays a significant role in lactate transport, then a saturation limit is expected (as shown by Jorfeldt et al., 1978). However, McLane and Holloszy (1979) reported that lactate uptake
by the resting rat hindlimb, increased linearly with increasing perfusate lactate concentration (between 6 and 26 mmol·L⁻¹).

More recent findings suggest that most intracellular lactate appears to move across cell membranes via the specific monocarboxylate transport system (carrier mediated efflux; Stainsby and Brooks 1990; Juel et al., 1988; Juel, 1991). This translocation takes place as lactate/H⁺ co-transport (or alternatively, as lactate/ OH⁻ exchange) and may account for up to 60% of the total efflux (Juel et al., 1988; Juel, 1991). Experiments in vitro have shown that muscle pH recovery occurs faster than lactate efflux, and this has been attributed to Na⁺/H⁺ exchange (Aickin and Thomas, 1977; Aickin, 1986; Juel et al., 1988). A significant point regarding muscle lactate and pH recovery is that they depend on lactate and pH gradients between muscle and blood, with high [lactate] and [H⁺] in the blood reducing lactate and H⁺ efflux from the muscle (McCartney et al., 1983c; Renaud, 1989; Juel et al., 1994). These findings would suggest that an increased blood flow to the exercising and/or the recovering muscle may be important for maintaining a high intracellular to extracellular lactate gradient (Gladden, 1989).

2.3.3. Muscle buffering capacity

The magnitude of decrease in muscle pH immediately after intense exercise is determined by the degree of lactic acid accumulation and the ability of the muscle to (a) buffer the free H⁺ and (b) to release H⁺ in the circulation.

The buffering capacity (β) of a solution, defined by Van Slyke in 1922 (in Hultman and Sahlin, 1980), is the amount (mmol·L⁻¹) of free H⁺ or OH⁻ required to produce a change in pH of 1 unit. It expresses the tendency of a solution to resist a change in pH following addition of a strong acid or base, more effectively than an equal volume of water (Martin et al., 1983). The unit for buffering capacity is the Slyke (mmol·pH⁻¹·L⁻¹).

Buffering of H⁺ in the muscle can be divided into two main types (Hultman and Sahlin, 1980):

- **Physicochemical buffering**, involving proteins (histidine residues), dipeptides (carnosine, anserine), phosphates and the CO₂/bicarbonate system
• **metabolic buffering**, involving mainly PCr breakdown, and in a much lesser degree IMP accumulation.

Another, more functional, categorization of buffering has been used by Harris *et al.* (1990). Thus, **static buffers** include proteins, dipeptides and phosphates, while **dynamic buffering** is done by the CK/PCr and the CO₂/bicarbonate systems.

The efflux of H⁺ from the muscle, is not considered as an intramuscular buffer, and has been discussed in the previous sections.

The ability of static buffers to buffer H⁺ depends on their concentration (content) and their pK values. Only those with a pK value within the physiological pH range will be effective. Thus, proteins can buffer H⁺ due to the pK of histidine residues (6.0-6.8; Dawson *et al.*, 1969; Hultman and Sahlin, 1980). Similarly, the dipeptide carnosine has a pK of 6.83 at 37 °C (in Harris *et al.*, 1991b). Finally, phosphate containing compounds (e.g. ATP, ADP, Pi, glucose-1-phosphate, fructose-6-phosphate) all have pK's between 6.1 and 7.0. The only exception is PCr, which has a pK of 4.6. However, muscle activity (ATP/PCr utilisation) results in Pi formation from PCr (pK: ~6.8), and thus PCr breakdown is said to 'consume' H⁺. The stoichiometry of hydrogen ions in the CK reaction is dependent on muscle pH. The stoichiometric coefficient can be calculated from pH and pK values (see APPENDIX E), and is 0.38 at pH 7.0 and 0.70 at pH 6.4 (Fig. 2.11). It is very interesting that the muscle fibres with the higher glycolytic capacity (fast twitch) have a higher content of phosphocreatine (Tesch *et al.*, 1989). This reflects their higher capacity for metabolic buffering.

The contribution of physicochemical buffering to the total cellular buffering, depends on the uptake of H⁺ by weak bases. The major contribution (~51%) to physicochemical buffering is made by muscle proteins, while the CO₂/bicarbonate system (~30%), the free inorganic phosphate (~13%) and the imidazole-containing compound carnosine (~6%) also contribute (Hultman and Sahlin, 1980; Parkhouse and McKenzie, 1984; Sahlin *et al.*, 1978a). The total amount of H⁺ taken by physicochemical buffering during exhaustive bicycle exercise, was estimated by Hultman and Sahlin (1980) to be 24.4 mmol.l⁻¹ muscle water (~61% of total buffering capacity).

Buffering by the CO₂/bicarbonate system has a significant contribution to pH control when the circulation to the muscle is not restricted.
Fig. 2.11. Stoichiometric coefficient for $H^+$ uptake during PCR utilisation at different muscle pH (for calculations, see APPENDIX E).

Approximately 70% of muscle bicarbonate is lost during exhaustive exercise (Sahlin et al., 1978a). Recently, Kowalchuck et al., (1988a, 1988b) have shown that after a 30 s all out sprint on an isokinetic cycle ergometer, the intramuscular acidosis is initially countered mainly by the rapid elimination of CO$_2$ by the lungs. This demonstrated the important role of the lungs and circulation in eliminating the CO$_2$ produced after buffering of $H^+$ by the bicarbonate system:

$$H^+ + HCO_3^- \leftrightarrow CO_2 + H_2O$$

When buffering capacity is determined by titration, only the static component is measured because PCr and ATP are almost completely lost during homogenization (Spriet et al., 1986; Marlin and Harris, 1991). Moreover, if the sample is freeze-dried, bicarbonate is also lost (Harris et al., 1989; Marlin and Harris, 1991). It has been suggested that titration of muscle homogenates is best to be performed over the pH range 7.1-6.5 due to the non-linear response from pH 7.0 to pH 6.0 (Harris et al., 1990). This
range represents that usually observed in muscle in the transition from rest to fatigue (Hultman and Sahlin, 1980).

The contribution of dynamic buffering processes can be estimated by combining titration measurements ($\beta_{\text{titr}}$) with 'in vivo' buffering capacity values ($\beta_{\text{vivo}}$) calculated from changes in muscle lactate and muscle pH. Marlin (1989) reported that $\beta_{\text{vivo}}$ was 63% higher compared to $\beta_{\text{titr}}$ in the horse. However, Mannion et al., (1993) found only a small ($\approx 8\%$) difference between the two, and suggested that this was probably an artifact resulting from a combination of factors affecting the measurement of muscle pH and in muscle homogenates.

The value of ($\beta_{\text{titr}}$) for human muscle is in the range of 44.7-67.6 Slykes (Sharp et al., 1986; Nevill et al., 1989), and measurements over the pH range 7.1-6.5 give similar results (135-170 mmol $\text{H}^+\cdot\text{kg dry muscle}^{-1}$; Harris et al., 1990; Mannion et al., 1993, 1994). Muscle buffering capacity is higher in trained individuals (Sharp et al., 1983; Sahlin and Henriksson, 1984; Parkhouse et al., 1985), and while short term-high intensity training has been found to increase $\beta_{\text{vivo}}$ (Sharp et al., 1986; 36% improvement in 8 wk) no change has been observed in $\beta_{\text{titr}}$ despite the improved sprint exercise performance (Nevill et al., 1989; Mannion et al., 1994; training lasted 8 wk and 16 wk, respectively).

In humans, carnosine concentration is not high (compared with the horse), and therefore does not contribute significantly to muscle buffering (Harris et al., 1990; Mannion et al., 1992). The most important buffers for human muscle in vivo are PCr breakdown (37% of total buffering capacity) and proteins (34% of total buffering capacity). Phosphates contribute $\approx 11\%$ and the $\text{CO}_2$/bicarbonate system $\approx 13\%$ (recalculated from Hultman and Sahlin, 1980).

### 2.4. RECOVERY FROM SHORT-TERM INTENSE EXERCISE

#### 2.4.1. Resynthesis of Phosphocreatine in muscle

Immediately after high intensity exercise, the content of phosphocreatine in the working muscles is very low (see Table 2.1). Phosphocreatine resynthesis is a rapid process during recovery from intense exercise. The time course of PCr resynthesis after exhaustive dynamic exercise (8.7 min cycling at 60 revs $\cdot$ min$^{-1}$) and isometric knee extension (40-55 s at 66% of maximum
voluntary force) has been examined by Harris et al. (1976), by taking repeated biopsy samples during recovery. The data have been modelled using a two component exponential equation (fast and a slow recovery component), allowing the calculation of half-times, time-constants and rate constants for PCr resynthesis (see Table 2.2 and footnote for explanation). While no difference was found between the half time of the ‘fast’ component of PCr resynthesis after isometric and dynamic exercise (≈30-32 s), the amount of PCr resynthesised after 2 and 4 min of recovery was lower following isometric exercise.

It has been suggested that the initial fast phase of PCr resynthesis is limited by the availability of oxygen, whereas the subsequent slow phase is limited by the hydrogen ion transport out of the muscle (Sahlin et al., 1979). These authors (Sahlin et al., 1979) have observed a significant restoration of PCr (≈68%) when muscle samples were incubated for 15 min in a high oxygen atmosphere, while no recovery of PCr occurred during incubation in nitrogen. Thus, an oxygen dependent mechanism for the initial PCr restoration was hypothesised, which was linked to the activity of the mitochondrial isoenzyme of creatine kinase and demonstrated the significance of oxidative phosphorylation for PCr resynthesis. In support of these suggestions, Blei et al. (1993) reported that a reduction in oxygen delivery to the muscle by application of external pressure (as low as 10 mmHg) may limit PCr resynthesis. Furthermore, Idstrom et al., (1985) reported that the rate of PCr resynthesis in perfused rat muscle preparation after electrical stimulation, was correlated with oxygen delivery to the tissue. This evidence links the process of PCr resynthesis with muscle blood flow and oxygen supply. The importance of the circulation during recovery has been demonstrated by the finding that no PCr resynthesis occurred when blood flow to the leg muscles was occluded after fatiguing contractions. (Harris et al., 1975, 1976).

Our knowledge of muscle metabolism during recovery from exercise in humans has been limited by the invasive nature of muscle biopsy. Phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) has made it possible to perform repeated non-invasive determinations of intracellular phosphorus metabolites and muscle pH (Radda and Seeley, 1979; Meyer et al., 1982; Radda, 1986). Although a disadvantage of the method is that exercise is performed with small muscle groups (because of the size of the
magnet), very useful information about PCr resynthesis has been provided under many different conditions (e.g. submaximal and maximal exercise, before and after training; see Table 2.2). Several authors have suggested that PCr resynthesis can be used as a measure of the oxidative capacity of the muscle (Chance et al., 1981; Sjoholm et al., 1983; McCully et al., 1991, 1994). The fact that the initial rate of PCr resynthesis is not affected in patients with glycolytic enzyme deficiencies (no lactate and H+ production) but is slower in patients with peripheral vascular disease or mitochondrial myopathies (Chance et al., 1982; Radda et al., 1982; Radda, 1986) provides good evidence that PCr resynthesis is dependent on aerobic metabolism and the initial rate of resynthesis is not affected by low muscle pH (Sapega et al., 1987). Muscle pH probably affects the final 'level' of PCr resynthesis attained, through the effect of H+ on the creatine kinase equilibrium (Sahlin et al., 1975; Harris et al., 1977). However, Adams et al. (1991) reported that hypercapnic acidosis in cat muscle did not cause a shift in PCr equilibrium, as long as sufficient oxygen was delivered to the tissue.

The results from the \(^{31}\text{P}-\text{MRS}\) studies presented in Table 2.3, reveal that the higher values for half time of PCr resynthesis (slower resynthesis rates) are observed when pH is low. However, this occurs when the intensity of prior exercise is high, and it may be that other effects, related to exercise intensity, also contribute to the slower PCr resynthesis rates.

An important consideration when examining the recovery of PCr following intense exercise is the possible difference in PCr resynthesis between the two major fibre types (fast and slow twitch). Although PCr is decreased to a similar extent in both fibre types following a maximal 30 s sprint on a non-motorised treadmill (Greenhaff et al., 1994), there is evidence to suggest that PCr resynthesis may be slower in the fast, compared with the slow twitch fibres. In a study by Tesch et al. (1989) PCr was resynthesised to 50% (fast twitch) and 68% (slow twitch) of the resting level, 60 s after 30 maximal voluntary knee extensions. Similarly, PCr was higher in the slow twitch fibres 1 min after =83 s of electrical stimulation of the quadriceps at 20 Hz (Soderlund and Hultman, 1991). These differences between fibres were assumed to be related to the higher mitochondrial density and capillarization of the slow twitch fibres (Soderlund and Hultman, 1991). Very interesting results regarding muscle fibre composition, intracellular pH and PCr resynthesis in humans have been reported by Mizuno et al. (1994). In that study subjects performed
CHAPTER II: Review of Literature

Table 2.3. Time constant, rate constant and half time for phosphocreatine resynthesis following exercise.

<table>
<thead>
<tr>
<th>References</th>
<th>Subjects</th>
<th>EXERCISE</th>
<th>Type</th>
<th>Intensity</th>
<th>Duration</th>
<th>Tc (s)</th>
<th>K_rate (per min)</th>
<th>t1/2 (s)</th>
<th>muscle pH</th>
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<td></td>
</tr>
<tr>
<td>a) MUSCLE BIOPSY STUDIES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Harris et al. (1976)*</td>
<td>5M</td>
<td>isometric</td>
<td>66% MVC</td>
<td>40-55 s</td>
<td>32</td>
<td>1000</td>
<td>1.86</td>
<td>0.06</td>
<td>22, 690</td>
</tr>
<tr>
<td>4M</td>
<td>Cycling</td>
<td>60 revs·min⁻¹</td>
<td>8.7 min</td>
<td>30</td>
<td>250</td>
<td>1.98</td>
<td>0.24</td>
<td>21</td>
<td>173</td>
</tr>
</tbody>
</table>

b) ³¹P-MRS HUMAN STUDIES

<table>
<thead>
<tr>
<th>References</th>
<th>Subjects</th>
<th>EXERCISE</th>
<th>Type</th>
<th>Intensity</th>
<th>Duration</th>
<th>Tc (s)</th>
<th>K_rate (per min)</th>
<th>t1/2 (s)</th>
<th>muscle pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blei et al. (1993)</td>
<td>7M, 4F</td>
<td>forearm</td>
<td>E</td>
<td>1 Hz</td>
<td>128 s</td>
<td>63</td>
<td>0.95</td>
<td>44</td>
<td>7.00</td>
</tr>
<tr>
<td>McCully et al. (1993)</td>
<td>4</td>
<td>calf, PF</td>
<td>1 PF per 6 s</td>
<td>5 min</td>
<td>31</td>
<td>1.94</td>
<td>21</td>
<td>7.00</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>calf, PF</td>
<td>1 PF per 6 s</td>
<td>5 min</td>
<td>57</td>
<td>1.06</td>
<td>39</td>
<td>7.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McCully et al. (1991)</td>
<td>4M</td>
<td>calf, PF</td>
<td>1 PF per 4 s</td>
<td>5 min</td>
<td>25</td>
<td>2.36</td>
<td>18</td>
<td>7.00</td>
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<td></td>
</tr>
<tr>
<td>McCully et al. (1994)</td>
<td>5M</td>
<td>calf, PF</td>
<td>1 PF per 4 s</td>
<td>5 min</td>
<td>36</td>
<td>1.67</td>
<td>25</td>
<td>7.05</td>
<td></td>
</tr>
<tr>
<td>5M</td>
<td>calf, PF</td>
<td>&lt;1 PF s⁻¹</td>
<td>64 s</td>
<td>68</td>
<td>0.88</td>
<td>47</td>
<td>6.62</td>
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<td></td>
</tr>
<tr>
<td>Mizuno et al. (1994)</td>
<td>12M, 2F</td>
<td>wrist</td>
<td>F</td>
<td>inc, 1 F per 6 s</td>
<td>8 min</td>
<td>99</td>
<td>0.61</td>
<td>68</td>
<td>6.78</td>
</tr>
<tr>
<td>Yoshida (1993a)</td>
<td>6M</td>
<td>knee</td>
<td>F</td>
<td>50 F·min⁻¹</td>
<td>4 min</td>
<td>27-40</td>
<td>2.22-1.50</td>
<td>19-28</td>
<td>6.8-6.5</td>
</tr>
<tr>
<td>&amp; Watari (1993b)</td>
<td>11M</td>
<td>knee</td>
<td>F</td>
<td>50 F·min⁻¹</td>
<td>2 min</td>
<td>5</td>
<td>long distance runners</td>
<td>25</td>
<td>2.37</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>untrained subjects</td>
<td>37</td>
<td>1.62</td>
</tr>
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</table>

C) ³¹P-MRS ANIMAL STUDIES

<table>
<thead>
<tr>
<th>References</th>
<th>Subjects</th>
<th>EXERCISE</th>
<th>Type</th>
<th>Intensity</th>
<th>Duration</th>
<th>Tc (s)</th>
<th>K_rate (per min)</th>
<th>t1/2 (s)</th>
<th>muscle pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kushmerick &amp; Meyer (1985)</td>
<td>E, rat gastrocnemius</td>
<td>2-10 Hz</td>
<td>1.8 min</td>
<td>30</td>
<td>2.00</td>
<td>21</td>
<td>6.5-6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyer (1988)</td>
<td>E, rat gastrocnemius</td>
<td>0.25-0.75 Hz</td>
<td>8 min</td>
<td>86</td>
<td>0.69</td>
<td>60</td>
<td>6.8-7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyer (1989)</td>
<td>E, rat gastrocnemius</td>
<td>0.25-0.75 Hz</td>
<td>8 min</td>
<td>Control</td>
<td>83</td>
<td>0.73</td>
<td>57</td>
<td>6.8-7.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 wk Creatine depletion; total Cr↓ by 42%</td>
<td>52</td>
<td>1.16</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 wk Creatine depletion; total Cr↓ by 67%</td>
<td>28</td>
<td>2.13</td>
<td>20</td>
</tr>
</tbody>
</table>

T_c, time constant; K_rate, rate constant, t1/2, half time of PCr resynthesis; muscle pH: at the end of exercise; M, males; F, females; * two component exponential equation, where f 'fast' and s 'slow' component; e, to exhaustion; E, electrical stimulation; Y, young (28±7 y); O, elderly (66±6 y); PF, plantar flexion; F, flexion; inc, incremental; from Sahlin et al. (1975); from Sahlin et al. (1976); § high pH signal data for the subjects, reported in a companion paper, Yoshida and Watari (1993c);

Rate constant, time constant and half time were calculated from exponential curve fits on the data. The rate constant is equal to the fraction of phosphocreatine which would be resynthesised per min, if the velocity resynthesis did not fall off (Lowry and Passonneau, 1972). Calculation of the maximum resynthesis rate (in mmol·kg dry muscle⁻¹) requires the resting value (max rate=resting value x rate constant), which was not always quantified in the ³¹P-MRS (magnetic resonance spectroscopy) studies.
graded forearm exercise for 8 min, and muscle metabolism was monitored during exercise and recovery by $^{31}$P-MRS. Furthermore, muscle biopsies were taken to assess muscle fibre composition of the exercising muscles. A main finding was that the percentage of slow twitch fibres was negatively correlated with the half time for PCr resynthesis. Additionally, the subjects were divided into three groups based on the characteristics of Pi peaks seen in their $^{31}$P-MRS spectra during exercise (see Table 2.2). Splitting of the Pi peak has been previously shown to reflect the responses of different fibre types (Vandenborne et al., 1991). Indeed, comparison of the histochemical and $^{31}$P-MRS data showed that fibre type distribution agreed with classifications using the Pi peak(s).

As seen in section 2.3.3., PCr breakdown is a process that consumes protons ($H^+$). When PCr is resynthesised, the opposite phenomenon occurs, i.e. protons are liberated. It can be calculated that resynthesis of $\approx 55$ mmol PCr·Kg dry muscle$^{-1}$ at a pH of 6.4 (post-exercise), will result in the formation of $\approx 39$ mmol $H^+$·Kg dry muscle$^{-1}$, during the first minute of recovery (Sahlin, 1978). This accounts for the additional decrease of pH during the first minute of recovery observed in several studies (Metzger and Fitts, 1987; Baker et al., 1992; Yoshida and Watari, 1993a), or the initial stabilisation of pH, even though a rapid $H^+$ efflux occurs (Sahlin, 1978).

### 2.4.2. Recovery of muscle pH after high intensity exercise

As discussed before, muscle pH drops to values of $\approx 6.70$ after a 30 s all out sprint on a non-motorised treadmill (Cheetham et al., 1986; Cheetham, 1987; Nevill et al., 1989), while it can decrease to 6.50 after repeated 30 s bouts of maximal sprint exercise (Spriet et al., 1989).

The recovery of muscle pH after intense exercise is a slow process, in comparison with PCr restoration (half time for pH $>3.6$ min; Sahlin et al., 1976). Until recently, the invasive nature of muscle biopsy technique limited the number of observations that could be made, in order to follow the pH changes in the muscle after exercise. Allsop et al. (1990), used a needle-tipped pH electrode which allowed monitoring of the changes in extracellular pH after a maximal 30 s sprint on a non-motorised treadmill. Muscle pH decreased from 7.17 at rest to 6.57 immediately after the sprint. One main finding of the study was that after an initial increase of pH to
6.70 (2 min into recovery), no further improvement was seen until after 10 min. This plateau in muscle pH observed between the 2nd and 10th min. of recovery is in marked contrast to the immediate increase in muscle pH found after cessation of exercise by Hermansen and Osnes (1972), using the homogenate technique on muscle biopsy samples. Allsop et al. (1990) suggested that this difference may reflect the diversity between the extracellular (pH electrode) and the intra- plus extra- cellular muscle pH (homogenate technique).

However, data from \(^{31}\text{P-MRS}\) studies have shown that intracellular pH recovery is indeed a slow process. For example in a study by Baker et al. (1992) intracellular pH dropped to \(~6.5\) after 2 min of maximum voluntary contraction of the ankle dorsiflexors. A further decrease of pH was seen during the first min of recovery (to 6.4), and pH reached only 6.8 after 6.5 min of rest. As argued previously when discussing PCr recovery, the pH restoration may also be affected by muscle blood flow.

2.4.3. Muscle blood flow and recovery of muscle metabolites

Relatively little information exists regarding muscle blood flow during recovery from high intensity exercise. Various techniques have been used to measure muscle blood flow, including clearance of tracers (Bonde-Petersen et al., 1975), and thermodilution methods (Andersen and Saltin, 1985). Experiments using exercise of a small muscle mass, have shown that the capacity of the vascular bed for blood flow is much larger than perfusion during maximal exercise (Andersen and Saltin, 1985).

An adequate muscle blood flow is a prerequisite not only during exercise, but also during recovery. In the case of high intensity exercise where muscle metabolites change dramatically, the importance of blood flow may be greater in order to remove lactate and \(\text{H}^+\) from the muscle, and provide oxygen for the resynthesis of PCr (see Fig. 2.12). The magnitude of muscle blood flow is dependent on mean arterial blood pressure (MABP), venous blood pressure (VBP), and local vascular resistance (LVR) according to the Haagen-Poiseuille equation (in Sjogaard 1987, 1990):

\[
\text{Flow} = (\text{MABP-VBP}) \times \text{LVR}^{-1}
\]
From this equation it is evident that blood flow increases when MABP increases or LVR decreases, while it decreases when VBP or LVR increase. These last two variables are in part dependent on the intramuscular pressure (the local VBP is assumed to equal intramuscular pressure). This theory shows that increases in intramuscular tissue pressure reduce muscle blood flow, which becomes zero when intramuscular pressure equals MABP.

During the recovery period following intense exercise, the increased blood pressure (increased during exercise) drops quickly (Miles et al., 1987; Sjogaard, 1987). If the recovery is passive, i.e. there is no muscle contraction, the highly beneficial effect of 'muscle pump' on local circulation is lost (Laughlin and Armstrong, 1985; Rowell, 1993).

![Simplified diagram showing some of the actions of increased blood flow during recovery from sprint exercise. PCr, phosphocreatine, La\(^{-}\), lactate.](image)

The actions of the 'muscle pump' are (Rowell, 1993):

- muscle contraction empties the veins, momentarily blocks arterial inflow and greatly increases venous driving pressure back towards the heart (increased venous return→increased stroke volume)
- Immediately after contraction, pressure in emptied veins falls to zero (valves momentarily prevent back flow and back pressure) and arterial-to-venous driving pressure is momentarily raised to a very high value.
In addition to the above factors, postural changes can affect muscle blood flow (and possibly recovery of muscle metabolites) due to hydrostatic effects, and can act in combination with the lack of the 'muscle pump' (Rowell, 1993). Spriet et al. (1989) postulated that the slow PCr resynthesis observed after repeated 30 s sprints on an isokinetic cycle ergometer was the result of 'pooling of the blood' in the legs due to the passive recovery on the bicycle seat between sprints. According to the above arguments, an 'active' recovery may increase blood flow to the previously exercised muscles by (a) 'muscle pump action' and (b) increasing the driving pressure of the blood towards the muscles, as a result of higher cardiac output.

2.4.4. Force and power restoration after fatiguing contractions

Experiments in vitro using animal muscle fibres have shown that during recovery from fatiguing contractions, force appears to recover faster than the disappearance of lactic acid. Metzger and Fitts (1987) argued that the initial, fast phase of recovery appears to be linked to a non- H+ mediated event, whereas the slow phase of force recovery is, at least, partially linked to a H+ mediated disruption in excitation-contraction coupling.

Similar biphasic patterns of force recovery have been observed by Sahlin and Ren (1989) after isometric knee extension to fatigue (~52 s at 66% of maximum voluntary force). They found that maximum voluntary force (knee extension) was completely restored after 2 min of recovery, despite the high muscle lactate (Sahlin and Ren 1989). It has been argued that the recovery pattern of power output follows the time-course of PCr resynthesis. The few studies examining the recovery of maximal power output after exercise (submaximal or slightly above maximal intensities: 60-120% VO2max), have suggested that PCr resynthesis may play a dominant role in maximal power output recovery (Sargeant and Dolan, 1987; Hitchcock, 1989). However, this conclusion was based on speculation and not on measurements of muscle metabolites.

The recovery of power output has been shown to be related to muscle characteristics such as fibre type and capillary supply. The relationship between capillary supply of the quadriceps femoris muscle and recovery of power output after short-term maximal isokinetic knee extension exercise (Tesch and Wright, 1983), gives further support for the significance of the circulation during recovery. Furthermore, individuals with a higher
percentage of fast twitch fibres fatigue faster and recover slower during repeated maximal knee extension exercise (3x30 extensions, with 60 s recovery; Colliander et al., 1988).

2.5. METABOLIC ASPECTS OF FATIGUE DURING HIGH INTENSITY EXERCISE

2.5.1. Sites of fatigue during high intensity exercise

Following short-term intense exercise, fatigued muscle exhibits impaired contractile performance, characterised by decreases in the maximal contraction velocity, peak tension, and rate of ATP hydrolysis. The basic mechanisms and the physiological sites involved in fatigue are complex. Edwards (1981, 1983) defined fatigue as "the failure to maintain the required or expected power output or force", and distinguished between central and peripheral fatigue. Central fatigue was attributed to impaired motivation (e.g. pain or perception of excessive effort) and reflex drive, and had its origin in the central nervous system. Peripheral fatigue refers to impaired activation/excitation of motor units, energy supply, and actin-myosin cross bridge coupling (see Fig. 2.13). Edwards (1983) finally suggested that fatigue is local in origin, with the brain simply modifying local events. Recently, an increased emphasis has been given to the central basis of fatigue (Jones and Bigland-Ritchie, 1986). It has been suggested that most human muscles can be fully activated by voluntary effort, but maximum force can only be sustained by highly motivated and experienced subjects in the presence of visual feedback (Bigland-Ritchie et al., 1986; Jones and Bigland-Ritchie, 1986). Physiological mechanisms which are designed to be protective, can be "overridden" in athletes and "underridden" in people who can not or will not activate their muscles fully (Edwards, 1983). Similarly, a feeling of discomfort due to intense exercise can "lower" the threshold of fatigue. Although central fatigue plays a role in "the inability to sustain power or force", very little information exists on its contribution during high intensity exercise. Much more attention has been directed towards the metabolic basis of fatigue (Hermansen, 1981; Vollestad and Sejersted, 1988).
Fig. 2.13. Sites of peripheral fatigue: (1) cell membrane, (2) t-tubular charge movement, (3) mechanism coupling the t-tubular charge movement with Ca\(^{2+}\) release, (4) Ca\(^{2+}\) release, (5) Ca\(^{2+}\) reuptake by sarcoplasmic reticulum, (6) Ca\(^{2+}\) binding to troponin, (7) actomyosin ATP hydrolysis, cross-bridge force development, and cycling rate (in Fitts and Metzger, 1988)

The decline of contractile force of skeletal muscle that occurs with fatigue is associated with a decrease in intracellular levels of PCr and ATP, an increase in inorganic phosphate (Pi) and its acidic fraction (H\(_2\)PO\(_4^-\)), H\(^+\), and ADP, and the consequent decrease in free energy available from ATP hydrolysis (Godt and Nosek, 1986). All of these changes have been suggested as possible fatigue-inducing agents (Fitts and Metzger 1988; MacLaren et al., 1989).

Many studies have reported that the rate of ATP resynthesis declines at approximately the same rate as the force generation (Hultman et al., 1990; Soderlund, 1991). This can be interpreted in two ways: either (i) that a decreased rate of ATP resynthesis results in a reduced force and thus is responsible for fatigue (i.e. failure of the energy systems to maintain a given rate of ADP rephosphorylation), or (ii) that force development decreases due to other factors (e.g. accumulation of metabolites), resulting in a lower rate of ATP
hydrolysis simply because the energy demand has dropped (Hultman et al., 1990).

The fact that ATP rarely drops below ~50% of the resting value during very intense exercise (Boobis et al., 1983; Harris et al., 1991) in combination with the very low $K_m$ of myosin ATPase for ATP (Glyn and Sleep, 1985) has been taken as firm evidence that fatigue is not related to a reduced energy supply (Jones and Round, 1990). Therefore the accumulation of products of the ATP hydrolysis (Pi, ADP, H+) and decreased pH due to the large contribution of anaerobic glycolysis have been examined as potential fatigue factors in many studies.

2.5.2. Accumulation of Pi and ADP and H+

2.5.2.1. Inorganic phosphate and force generation

Numerous studies have been carried out focusing on the effects of inorganic phosphate, pH and ADP (Cooke et al., 1988; Kentish, 1986; Godt and Nosek, 1989; Curtin and Edman, 1994). Usually, the ‘skinned’ fibre preparation has been employed, and therefore stimulation of the fibre was achieved by manipulating $Ca^{2+}$ concentration of the bathing solutions. By ‘skinning’ the fibres only myosin ATPase could be studied. In the skinned muscle, modest levels of Pi (~10 mmol·l⁻¹ intracellular water) have a large effect on force generation (Kentish, 1986). However, much higher levels of Pi are sustained in exercising human muscle, with only a small decrease in force (Cady et al., 1989a). $^{31}P$-MRS studies have shown that the effects of inorganic phosphate on force production are potentiated when a decrease in muscle pH occurs (Wilkie, 1986; Wilson et al., 1988). This is because the pK of phosphate is ~6.8 and a shift in pH changes the proportions of the monobasic ($H_2PO_4^{-}$) and dibasic species ($HPO_4^{2-}$). Wilson et al. (1988) argued that accumulation of $H_2PO_4^{-}$ was the main cause of fatigue. However, the dominant role of $H_2PO_4^{-}$ was disputed by Cady et al. (1989a) who observed much less monobasic phosphate but rapid force loss in a subject with myophosphorylase deficiency (no $H^+$ production from glycolysis). Similarly, Adams et al. (1991) found a dissociation between tension (force) and $H_2PO_4^{-}$ or pH using hypercapnic acidosis on isolated cat muscle. Clearly there is an effect of Pi and $H_2PO_4^{-}$ on force, but it is not the only factor to induce fatigue. The mechanism by which Pi acts to inhibit force
Fig. 2.14. The main steps in the cross-bridge cycle and their probable relation to mechanical events. Force is generated between steps ii & iii. The role of ADP and Pi accumulation in the different steps of the cross bridge-cycle and its relation to fatigue is discussed in the text (in Jones and Round, 1990).

generation by product inhibition of the actomyosin ATPase is based on the biochemical event during the steps of actomyosin interaction, as follows (Jones and Round, 1990; see Fig. 2.14):

• The first step (i) involves attachment of actin and myosin, which will give stiffness to the muscle but does not generate force
• The release of phosphate from the actomyosin complex (ii) is thought to initiate the changes that result in rotation of the head of myosin, and therefore force generation
• Close to the end of the rotation phase ADP is released (iii) and then the actomyosin complex can bind another ATP which will allow dissociation of myosin from actin. This ATP is then hydrolysed and the products remain bound to myosin. ATP hydrolysis is thought to activate the myosin head, making it ready for the next cycle.

This model is useful in facilitating understanding of the differences in product inhibition of myosin ATPase by ADP and Pi.
2.5.2. Slowing of relaxation

One of the characteristic features of skeletal muscle fatigued by high force contractions is a slowing of the rate of relaxation from an isometric contraction. Two mechanisms can explain slowing of relaxation: (i) a reduced rate of cross bridge cycling in the fatigued state, due to a reduction in ATP or increase in ADP (Edwards et al., 1975) and (ii) reduced rates of Ca\(^{2+}\) reuptake by the sarcoplasmic reticulum ATPase. This process is ATP consuming (2 Ca\(^{2+}\) per ATP hydrolysed; Carafoli, 1991) and may be dependent on the free energy of ATP (Dawson et al., 1980). The effect of ADP accumulation on cross bridge cycling can be better understood when considering the steps of cross bridge cycling (see Fig. 2.14). Release of ADP occurs before cross bridge dissociation, and therefore an increased [ADP] near the myosin head will slow down ADP release. The involvement of products of myosin ATPase at different steps in the cycle may also explain the lack of force inhibition by high ADP (force is increased, rather than decreased; Cooke and Pate, 1990).

The involvement of hydrogen ions in the slowing of relaxation has been debated. Sahlin et al. (1981) did not observe slowing of relaxation when a muscle was fatigued after incubation with iodoacetic acid (IAA), which stops glycolysis at the glyceraldehyde - phosphate dehydrogenase step (thereby preventing a drop in pH). However, Edwards et al. (1975) found that IAA affected the rate of muscle relaxation. Cady et al. (1989b) reported that a subject with myophosphorylase deficiency (no H\(^{+}\) from glycolysis) showed an increased rate of relaxation during exercise, similar to that of normal subjects. However, the recovery of the relaxation rate was much faster in the subject with myophosphorylase deficiency, indicating the involvement of H\(^{+}\) in slowing of relaxation. Interestingly, recovery of relaxation rate showed a time course similar to that of PCr resynthesis, although a pH effect could be demonstrated (Cady et al., 1989b). A positive correlation between the recovery of relaxation rate and PCr resynthesis has also been reported by Sjoholm et al., (1983). The involvement of the Ca\(^{2+}\) pump in the slowing of relaxation, and the resemblance between the recovery kinetics of the relaxation rate and PCr resynthesis would suggest that the manifestations of fatigue may be related with energy supply mechanisms.
2.5.2.3 The role of H\(^+\) in muscular fatigue

The hydrogen ion is a particularly interesting potential fatigue agent, as it could cause fatigue by acting at numerous sites in the muscle, reducing both ATP utilisation and resynthesis. Briefly, increased [H\(^+\)] can:

- slow down glycogenolysis and glycolysis by inhibiting the two "key enzymes" PFK and phosphorylase.
- affect the 3 ATPases (myosin ATPase, Ca\(^{2+}\) reuptake ATPase, Na\(^{+}\)-K\(^{+}\) ATPase) by product inhibition, thereby slowing down relaxation rate and force, and impairing muscle membrane depolarisation (Cooke and Pate, 1990; Cooke et al., 1988).
- Compete with Ca\(^{2+}\) for the binding sites of troponin C, impairing contractile protein activation (Palmer and Kentish, 1994).
- Cause a reduced Ca\(^{2+}\) release from the sarcoplasmic reticulum (Nakamaru and Schwartz, 1972).
- increase the amount of free Ca\(^{2+}\) required for the myofilaments to develop a given tension (Fabiato and Fabiato, 1978; Godt and Nosek, 1986; Metzger and Moss, 1987).
- affect the creatine kinase reaction towards PCr breakdown (Sahlin et al., 1975, 1983), thereby reducing the high energy phosphates store.
- in conjunction with changes in ATP, ADP, Pi, free Mg\(^{2+}\), reduce the free energy liberated when one mole of ATP is hydrolysed.
- increase the formation of the 'acid form' of inorganic phosphate, thus reducing the substrate for phosphorylase (Chasiotis et al., 1982b) and force generation (Nosek et al., 1987).
- In a study by Metzger and Moss (1987), the effects of pH on contractile properties were investigated using single fibres from fast and slow twitch rat hindlimb muscles. The pH of the bathing solution was changed between 6.2 and 7.0, values similar to those observed in the pre- and post- fatigue condition following short-term intense exercise. The major finding was a marked depressive effect of H\(^+\) on maximum shortening velocity. In addition, force production and Ca\(^{2+}\) sensitivity of force development were altered by H\(^+\). An interesting observation was that slow twitch fibres were more resistant to changes in pH, in terms of altered function.

The effects of H\(^+\) accumulation have been separated from other metabolite changes in fatigued muscle, by blocking the glycolytic pathway using
iodoacetic acid (IAA; Sahlin et al., 1981). It has been demonstrated that electrical stimulation of unpoisoned rat muscle for 3 minutes, resulted in a decrease in pH in parallel with a decrease in tension development (to 50%), and an increase in relaxation time (to 250% of initial value). However stimulation of the IAA-poisoned muscle resulted in marked ATP depletion and rigor, without any decrease in pH. Thus it seems that a protective mechanism, associated with H⁺ accumulation acts as a "safety device" preventing the muscle cell from irreparable damage due to ATP depletion.

Clearly, fatigue in skeletal muscle can be induced without a change in pH, as shown in the experiments with myophosphorylase deficient patients or IAA poisoned muscle (Cady et al., 1989a, 1989b; Sahlin et al., 1981, 1990). However when dealing with an intact normal organism, the interaction between all the fatigue agents is important.

2.5.2.4. The role of calcium in fatigue

The role of calcium release from sarcoplasmic reticulum is central for muscle metabolism and function. Three possible cellular mechanisms have been identified, which link calcium and muscle fatigue (Westerblad et al., 1991; Allen et al., 1992):

- a decline in calcium release (which may be related to calcium reuptake and Ca²⁺ ATPase).
- a reduction of tension at saturating Ca²⁺ (e.g. effect of Pi on cross bridges)
- a decreased sensitivity of the contractile mechanism to Ca²⁺ (e.g. H⁺-Ca²⁺ competition for troponin; Palmer and Kentish, 1994).

The fact that the decline in tension of isolated fibres could be restored with caffeine (Nassar-Gentina et al., 1981; Westerblad et al., 1991) would suggest that fatigue may be mainly a phenomenon which occurs on the muscle membrane (and t-tubules) and not in the contractile mechanism itself.

2.5.2.5. The role of potassium in fatigue

Potassium has a vital role in muscle function. The excitability of muscle membrane is dependent on the membrane potential, which, in turn, is dependent mainly on the K⁺ gradients across the membrane (Sjøgaard, 1987). Consequently, the rise in extracellular potassium seen during high intensity exercise, will eventually cause a depolarisation block, and may be related to fatigue in that type of exercise (Vollestad and Sejersted 1988).
The loss of intracellular $K^+$ has been attributed to an impairment of the function of the $Na^+-K^+$ pumps ($Na^+-K^+\text{ATPase}$; Sejersted and Hallen 1987). It has been suggested that potassium might accumulate in the t-tubules, due to the existence of fewer $Na^+-K^+$ pumps at that site (Vollestad and Sejersted 1988) or due to a ‘local’ energy deficiency and cause depolarisation, reduced action potential amplitude and hence impaired voltage sensor activation (Westerblad et al., 1991).

Medbo and Sejersted (1994) reported that following an exhausting running bout (1 min), endurance-trained subjects had lower plasma [K+] during the recovery period compared with sprint-trained subjects, and it was suggested that the two groups of subjects may regulate $K^+$ differently. Regulation of $K^+$ in the blood and muscle has been shown to be improved by training. McKenna et al. (1993) found that sprint training for 7 wk reduced plasma $K^+$ in the recovery period following 4x30 s maximal sprints (4 min recovery) as a result of a 16% increase in the skeletal muscle $Na^+-K^+\text{ATPase}$ concentration.

**2.6. EFFECTS OF POWER-VELOCITY RELATIONSHIP ON POWER OUTPUT AND FATIGUE DURING SPRINT CYCLING**

2. 6. 1. Force and power-velocity relationships of human muscles

The force developed at different velocities of contractile-element shortening, describes a fundamental mechanical property of skeletal muscle. The force-velocity "curve" for skeletal muscle *in vitro*, was first described by Hill in 1938 as a rectangular hyperbola (in Green, 1986). In 1950, Wilkie reported that, after correction for inertia of the forearm, the force-velocity curve obtained for the elbow flexors could be represented by Hill's equation. However, when multi-joint movements are examined (e.g. during cycling), the force-velocity (F-V) relationship of the active muscles is modified according to the limb lever system (McCartney et al., 1985).

The power produced by a skeletal muscle is the product of the force it generates and the velocity at which it shortens. Since force is proportional to the cross sectional area of a muscle (Maughan et al., 1983), and velocity
CHAPTER II: Review of Literature

<table>
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<th>Pedal rate</th>
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<th>predicted maximum</th>
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\(+\text{revs.min}^{-1}\)

Fig. 2.15. Predicted power-velocity curves calculated from Hill's equation, for maximal pedalling speeds of 220, 260 and 320 revs.min\(^{-1}\) (in Lakomy, 1988).
to the length, it follows that power is proportional to the product of these, namely volume (Jones and Round, 1990). The power-velocity \((P-V)\) relationship of the muscle, can be calculated from Hill's equation and has a characteristic shape. Fig. 2.15 depicts the results of the calculations by Lakomy (1988) for cycling exercise, assuming three different maximum pedal speeds. Power reflects the interactions of force and velocity of a whole muscle (or joint(s)), under a given set of circumstances. The maximum force developed by a muscle is determined by the number of cross-bridges in the driving stroke (Jones and Round, 1990). According to Hill's calculations, peak power occurs at approximately \(\frac{1}{3}\) of the maximal force and \(\frac{1}{3}\) of the maximal, 'unloaded' velocity.

However, differences in power generation exist between the two major fibre types (i.e. slow and fast twitch fibres). While the force development per unit area of fast and slow fibres is similar, the shortening velocity of fast fibres is 3-4 times greater than that of slow fibres (Faulkner et al., 1986). This has also been shown for fast and slow rat muscle (Brooks et al., 1990b). Consequently, fast fibres can produce higher power at a higher shortening velocity (see Fig. 2.16), but are fatiguing faster. Slow fibres are "specialised" for protracted usage at relatively low velocities.

Compared with a 'pure' fast muscle, a mixed muscle composed of approximately 50% slow fibres, is at a considerable "disadvantage" in performing tasks requiring substantial power development. When all fibres in a mixed muscle are contracting, the slow fibres contribute as much power as the fast fibres at very low velocities, only slightly to power at moderate velocities, and not at all at high velocities (see Fig. 2.16; Faulkner et al., 1986).

The effect of muscle composition on power output has been demonstrated by McCartney et al. (1983b). They observed a 33% difference between the maximum power output of the "best" and "worst" cyclists. The "best" cyclist, who achieved his maximal peak power output at a crank velocity of 162 revs.min\(^{-1}\), had 72% of type II fibres, while the poorest one attained his maximum value at 119 revs.min\(^{-1}\), and had 53% of type II fibres.

Fatigue has been found to shift the force-velocity (and consequently the power-velocity) relationship of the muscle. Cooke et al., (1988) and Cooke and Pate (1990) using 'skinned' rabbit muscle in vitro, have demonstrated the effects of different metabolites related to fatigue (products of ATP
**Fig. 2.16.** Power output as a function of velocity for fast, slow, and mixed muscle. Power output curves are for pure fast and pure slow fibres (left), and for a mixed muscle (50% fast and 50% slow fibres; right). Power and velocity have been normalised by peak power and maximum velocity of shortening of the fast fibres. The shaded portion on the right graph represents the contribution of the slow fibres to power output of the mixed muscle (in Faulkner et al., 1986).

Hydrolysis: $\text{Pi, ADP, } H^+$ on the $F-V$ relationship. As can be seen in Fig. 2.17, elevated $H^+$ (pH: 7.0→6.0) caused substantial decreases in both maximal velocity (27%) and maximal force (46%). However, inorganic phosphate did not affect the maximum velocity of contraction, but only force. This finding has also been reported for intact fibres (frog muscle) during electrically stimulated contractions (Curtin and Edman, 1994), and can be explained by cross bridge kinetics. According to the model of cross bridge cycling presented earlier, $\text{Pi}$ release initiates force generation and therefore accumulation of $\text{Pi}$ will slow down this process. On the other hand $\text{Pi}$ is not involved in the step of cross bridge dissociation, and therefore can not affect the velocity of shortening (Jones and Round, 1990). Likewise, accumulation of $\text{ADP}$ will decrease maximum velocity, but not force (Cooke and Pate, 1990).

It has been suggested that when the muscle is fatigued from short-term high intensity exercise, the fast fibres are 'selectively' fatigued in comparison with the slow twitch fibres (Beelen and Sargeant, 1991, 1993).
The suggestion of a selective fatigue of the fast twitch fibres, especially when exercise involves fast movements, would fit the calculations of Faulkner et al. (1986; Fig. 2.16). Since at faster speeds fast twitch (FT) fibres are predominantly recruited (or are functionally more involved; Fig. 2.16), then force and power are expected to be much lower at these speeds after fatigue, due to impaired function of FT fibres. Greenhaff et al. (1994) analysed muscle metabolites in single fibres following a maximal 30 s bout of sprint running. Although both fibre types were highly involved (as shown by glycogenolytic and PCR degradation rates), the rates of PCR and glycogen degradation were 25% and 64% higher in the fast twitch fibres. Similar results have been obtained during electrical stimulation of the quadriceps (50 Hz) with the circulation occluded, while the calculated ATP utilisation rate was twice as high in the fast twitch fibres (Greenhaff et al., 1993b). These data would suggest that when intense exercise is repeated, the metabolic environment in the fast twitch fibres will be less favourable for maximum power generation (lower pH due to higher glycogenolysis), especially when considering that capillary supply and PCR resynthesis in these fibres are lower compared with the slow twitch fibres (Tesch et al., 1989; Soderlund and Hultman, 1991).

Fig. 2.17. Effect of elevated [Pi] (left) and [H+] (right) on fibre contraction. Dashed curve from unfatigued muscle, and solid curve after elevation of Pi (left) and a decrease in pH (right), as would be observed in fatigued muscle (in Cooke and Pate, 1990).
2.6.2. Power-velocity relationship and sprint cycling exercise

It has been reported that the optimum pedalling velocity for maximal power generation during isokinetic cycling is $=110 \text{revs.min}^{-1}$ (Sargeant et al., 1981). Using a similar cycle ergometer, McCartney et al (1983b, 1985) found that during a 30 s sprint on an isokinetic cycle ergometer, peak power output was achieved at pedal velocities between 120 and 160 revs.min$^{-1}$, with a large inter-subject variation. A theoretical calculation of the power-velocity relationship from Hill's equation, has been presented in Fig. 2.15, for three assumed maximal pedalling speeds (Lakomy, 1988).

The shape of the power output profile during a 30 s sprint on a friction-loaded cycle ergometer, has led Lakomy (1988) to investigate the effects of P-V relationship on power output, during that type of exercise. The initial rapid decline in power output (within 1-3 seconds) measured with free accelerating ergometers was initially interpreted as a metabolic effect (Cheetham, 1987). However, Lakomy (1988) offered a "non-metabolic" explanation for this initial-rapid drop, which was related to P-V relationship.

Usually during a 30 s cycle ergometer sprint, a maximal pedalling velocity of 150-180 revs.min$^{-1}$ is observed after 3-5 s from the start of the test. As the subject accelerates the flywheel from the rolling start speed ($=70 \text{revs.min}^{-1}$), the power output increases until the individual 'optimum' pedalling speed is reached (see Fig. 2.15). This occurs at about the 1st-2nd second of the sprint. However, the subject continues to accelerate until the maximum speed is reached. Therefore, as the speed is increased over the "optimum point", the power drops due to the P-V relationship, and not to metabolic factors (see Fig. 2.15).

Once peak speed is reached, the pedalling rate starts to decline. If metabolic changes had not occurred, then power output would have started to rise as the pedalling speed declined towards the optimum value. Using this 'model', Lakomy (1988) calculated the contribution of the metabolic fatigue to the measured fatigue. The contribution of the metabolic fatigue to the fatigue during the acceleration phase was as low as 3% for a load of 55 g·Kg$^{-1}$ body mass, and 19% for a load of 95 g·Kg$^{-1}$ body mass.

The arguments presented in this section demonstrate the complexity of factors which have to be taken into account when attempting to interpret
fatigue during sprint exercise on a friction-loaded cycle ergometer (where speed of contraction changes). It is evident that both metabolic (biochemical) and purely mechanical factors (related to muscle torques, and P-V relationships) affect the 'external' power output.

2.6.3. Effects of muscle temperature on power generation during high intensity exercise

A factor which can modify power output generation independent of changes in muscle metabolites, is a change in muscle temperature. This effect is particularly relevant when sprint bouts are repeated, because the increase in temperature during successive sprints would tend to increase power output, while fatigue would decrease power output. Despite its relevance to sprint exercise, very few studies have systematically examined the effects of temperature on maximal power output. Sargeant (1987) manipulated leg muscle temperature by immersing both legs in water of varying temperatures, and observed changes in power output during a 20 s bout of maximal isokinetic cycle ergometer exercise. It was reported that an increase in the quadriceps muscle temperature from 36.3 °C (at rest) to 39.3 °C (warm water bath) resulted in a ~11% increase in peak power output when cycling at 95 revs·min⁻¹. Furthermore, the effect of temperature was found to be different at different cycling speeds. Thus, at the slowest pedalling rate (54 revs·min⁻¹) the increase in peak power was ~2% per °C while at the fastest (140 revs·min⁻¹) it was 10% per °C. Also the optimum velocity for power production increased as muscle temperature increased (from 109 revs·min⁻¹ at room temperature to 125 revs·min⁻¹ after 45 min immersion in a water bath at 44 °C; n=2; Sargeant, 1987).

Changes in quadriceps muscle temperature after 30 s of sprint running on a non-motorised treadmill have been monitored using a thermocouple on the tip of a needle inserted in the muscle (Allsop et al., 1990). The increase in muscle temperature after the sprint was 2.1 °C (35.8→37.9 °C), and the decline towards resting temperature was slow (~37 °C at the 10th min of recovery). If a second sprint were to be performed after a short recovery period, one would expect power output to be modified by the different muscle temperature (compared to that before the first sprint; Rademaker et al., 1994). In the study of Allsop et al. (1990) the warm-up consisted of only two 30 s runs at relatively low speeds (8 and 10 km·h⁻¹), separated by 5 min of rest. A more thorough warm-up procedure may be necessary to increase
muscle temperature before the first sprint and therefore minimise temperature effects on power output during repeated sprints.

2.7. SUMMARY

This review has shown that the high rates of ATP utilisation seen during sprint exercise result in a pronounced accumulation of metabolites such as Pi and H⁺, and PCr depletion. Although ATP itself may not decrease to levels that will impair muscle function, the rate of ATP utilisation is reduced during repeated maximal exercise. This finding has led to the conclusion that fatigue is not related to energy supply mechanisms, but is the result of product inhibition and metabolic control. Although the weight of the available evidence suggests that accumulation of metabolites may control muscle function, the role of the energy systems (i.e. PCr and glycolysis) may be important. The immediacy and effectiveness of PCr to remove products of the ATPases (ADP and H⁺) would suggest that [PCr] is important not only as a source of energy, but also as a means to reduce product accumulation for all three ATPases. The creatine supplementation studies have provided further support for the significance of PCr. An interesting point emerging from this review is that the major site of muscle function control (and thus site of fatigue) may lie outside the contractile mechanism (i.e. on the muscle membrane). However, the excitation-contraction coupling involves steps which are dependent on ATPase reactions, and thus influenced by rates of energy supply. The relationship between the recovery of power output and energy supply mechanisms requires further investigation.
CHAPTER III

GENERAL METHODS

3.1. INTRODUCTION

The methodology common to all studies presented in this thesis is described in detail in the six sections that follow. The first section (3.2.) describes the equipment and instrumentation used to measure the external power output generated by the subjects during maximal sprinting. The standardised procedures used during familiarisation and testing, and the performance variables measured and calculated can be found in the second section (3.3.) The procedures for collection and analysis of blood and muscle samples are described in the third and fourth sections (3.4., 3.5.), respectively. Finally, the fifth and sixth sections (3.6., 3.7.) contain calculations done using muscle metabolite data and the statistical techniques employed, respectively.

3.2. MEASUREMENT OF EXTERNAL POWER OUTPUT DURING SPRINT CYCLING

3.2.1. CYCLE ERGOMETER

Cycle ergometry has emerged as a popular power - testing mode, primarily because of its capacity to provide measures of various aspects of human power output (peak power, total work, fatigue etc.), during brief, all - out efforts.

In all the studies composing this thesis, a modified friction - loaded cycle ergometer (Monark, model 864), interfaced with a BBC (model B) microcomputer, was used in order to calculate the "corrected" instantaneous power, generated during maximal sprint exercise (Lakomy, 1986, 1988). In contrast with other methods used for calculation of the power output during cycle ergometry, which ignore the inertia characteristics and the instantaneous changes in angular velocity of the
flywheel (e.g. Wingate test, Bar-Or, 1978; Williams et al., 1988 etc.), this method enables correction for these factors.

Lakomy (1988) found that the errors resulting from the traditional method of calculation of peak power output (Bar-Or, 1978), can be as large as 32% (underestimation). However, if the 30 s test is done from a rolling start of about 70 revs·min⁻¹, then the mean power output calculated using the traditional "uncorrected" method is not significantly different from the "corrected" value. This similarity in mean power output is because the pedalling speed at the start of the test is similar to that at the end of the test and consequently, the work done during the acceleration phase resulting in the increased kinetic energy of the flywheel is recovered from the flywheel during the subsequent deceleration (Lakomy, 1988).

In order to obtain the "corrected" power output values, a high-speed system for data collection was used (Lakomy, 1986, 1988). It consisted of a small electric generator which was driven by the ergometer's flywheel, giving an analogue signal proportional to the angular velocity of the flywheel. This signal was logged by the microcomputer via an analogue-to-digital converter, along with a timing signal derived from the computer's internal clock. The sampling rate was 20 Hz. The new parameter calculated in the "corrected" method was termed by Lakomy (1988) the "acceleration-balancing load". Simply, this is the load that would be required to balance any torque attempting to accelerate the flywheel. It should be noted that the "acceleration-balancing load" incorporates not only factors resulting from the moment of inertia of the flywheel, but also compensates for a number of losses caused by the rolling resistance of the flywheel and freewheel mechanism. The values of instantaneous "corrected" power were therefore obtained from the product of the speed of the flywheel and the "effective" load (which is the resistive load plus the instantaneous "acceleration balancing load"). The values for instantaneous speed and power were averaged over 1 s intervals. At the conclusion of the test, the computer was programmed to calculate, display and print the test results. It must be emphasised that with the "corrected" method of external power measurement, the peak power is always reached before peak speed, which is what would be expected based on theory. A typical power output and pedal speed profile is shown in Fig. 3.1.
3.2.2. CALIBRATION PROCEDURES FOR THE CYCLE ERGOMETER

Before and at the end of each study, the relationship between flywheel speed and the output from the generator and analogue-to-digital converter were carefully calibrated. The ergometer was pedalled by one of the experimenters for 100 seconds, at approximately 60 revs·min⁻¹. The actual speed of pedalling was not important, nor was it required to be constant. Flywheel revolutions were counted using an electromechanical counter. The procedure of the calibration has been described in detail elsewhere (Lakomy, 1988).

Thereafter, the deceleration time of the flywheel was determined by setting the flywheel in motion, pedalling in excess of 105 pedal revs·min⁻¹, and then ceasing pedalling, using a series of resistive loads. From these trials, a linear regression equation of load vs flywheel deceleration was obtained ($r^2 \geq 99.9\%$), enabling the calculation of the “acceleration balancing load” (for details, see Lakomy, 1988).

![Graph showing power output and pedal speed profiles generated during a 30 s sprint on the modified cycle ergometer.](image-url)
3.2.3. THE USE OF A RESTRAINING HARNESS DURING MAXIMAL SPRINT CYCLING

Although the need to remain seated on the cycle ergometer during the sprint has been emphasised in almost all previous studies (e.g. Williams et al., 1988; Lakomy, 1988), only a few experiments have used physical restraints to minimise the contribution of body weight and upper body muscles to external power generation. A restraining harness passed around the subject's waist was employed by McCartney et al. (1983a, 1983b) during high intensity isokinetic cycling, in order to limit the exercise to the lower limbs. In the present study, the upper body muscles were "isolated" (subjects held the handlebars mainly for balance), and the position of the subject on the cycle ergometer was standardised by using a restraining belt, similar to that used by McCartney et al. (1983a). A wide tether belt (used in trampoline stunts) was passed around the subject's waist. The position of the belt was such, that thigh flexion was not restricted, and there was no pressure on the subject's stomach. The two side straps of the belt were fixed to a metal rail, bolted on the floor behind the bicycle frame. Holes were drilled along the metal rail and a small metal ring was used to adjust the tension of the straps for each subject. (see Fig. 3.2.). The optimal seat height was adjusted for each subject so that the knee was slightly flexed when the pedal was at the bottom of its travel. Toe clips (reinforced with adhesive tape) were used to secure the subject's feet on the pedals. When adjusting the tension of the harness, care was taken to standardise for the degree of backward pelvis tilt. By tilting the pelvis the length of the hip extensor muscles is changed, and as a result, these muscles may not work at their individual optimal shortening range (Wells and Luttgens, 1976; Visser et al., 1990).

The effects of the restraining harness on power output during sprint cycling have been examined previously by Bogdanis (1991). Peak power output was unaffected by the use of the restraining harness, and further pilot studies showed that peak power output may even increase slightly as a result of use of the harness after thorough familiarisation (2-3 sessions). This may be due to the fact that the harness holds the subject on the seat, allowing most of the power generated by the legs to be applied on the flywheel and not to lift the body off the seat (especially during instants of high acceleration/force application).
Fig. 3.2. The modified cycle ergometer and restraining harness used in this thesis.
3.3. FAMILIARISATION AND STANDARDISED TESTING PROCEDURES

3.3.1. SUBJECTS

All experimental tests in this thesis were conducted in the laboratory under neutral environmental conditions (18-23 °C). Subjects were informed in writing about the purpose of the study, any known risks, and the right to terminate participation at will. Each expressed understanding by signing a statement of informed consent. A short questionnaire was attached to that statement, in order to obtain information about the sporting/recreational activities of each subject. A medical history questionnaire was also completed in the presence of the experimenter, and subjects with medical problems were excluded. All subjects who took part in the studies were physically active, and some were involved in regular training. Each testing protocol was approved by the Ethical Committee of Loughborough University of Technology.

3.3.2. FAMILIARISATION

Prior to any experimental testing each subject completed 2-4 sprinting practice sessions. These sessions were considered as very important, since the subjects had to:

- get accustomed to all-out sprinting, wearing the restraining harness.
- learn to accelerate maximally at the command "go", (very important in order to attain the 'true' individual peak power output).
- get familiarised with exerting maximal effort during repeated sprint cycling

The first familiarisation session consisted of: warm up on the cycle ergometer; 3-4 brief sprints (= 6 s) without the harness, aiming to attain a high peak power output; 3-5 brief sprints with the restraining harness.

The second session was similar to the first (harness on from the start), and the third session involved two 20 s - 30 s sprints separated by 2-4 min (depending on the main study).

All the results from these tests were recorded and kept to monitor improvement with each familiarisation session, and to check if the subjects gave a maximal effort during the experimental tests. During the
familiarisation sessions the optimal seat height and restraining harness arrangement, were determined for the future tests. All this information, together with body height (Holltai stadiometer) and body mass measurements (Avery 3306 ABV balance), were recorded on individual data sheets.

3.3.3. DIET AND EXERCISE CONTROL

Subjects were requested to repeat their pre-recorded normal diet and refrain from any form of intense physical exercise for 48 hours prior to each test. Each subject performed all tests at the same time of day, which was at least four hours after any meal.

3.3.4. PRELIMINARY MEASUREMENTS

3.3.4.1. Determination of maximal oxygen uptake

The maximal oxygen uptake of each subject (\( \dot{V}O_2^{\text{max}} \)) was determined during the second or third preliminary visit (studies ii, iii, iv), using a continuous incremental test on the Monark 814 cycle ergometer. Subjects performed a 2 min warm-up at 60 W and then work rate was increased by 30-60 W every 3 min until exhaustion (~11-14 min). Strong verbal encouragement was given to each subject during the test. Pedalling frequency was kept at 1 Hz (60 revs·min⁻¹) throughout the test. Expired air was collected during the last min of each stage (1' 45"- 2' 45") using the Douglas bag technique. Heart rate was monitored throughout the test using short range telemetry (Sports Tester, Polar Electro Fitness, PE3000), and perceived rate of exertion was recorded every three minutes (Borg, 1982). The \( \dot{V}O_2 \) value obtained during the last expired air sample was taken as the \( \dot{V}O_2^{\text{max}} \) value.

Criteria for attaining the "true" \( \dot{V}O_2^{\text{max}} \) included (in addition to volitional exhaustion) a respiratory exchange ratio (R) > 1.10; only a small or no increase in \( \dot{V}O_2 \) from the penultimate to the last expired air sample (<0.15 l·min⁻¹; Taylor et al., 1955); heart rate = 220 - age (± 10 beats·min⁻¹); ventilatory equivalent for oxygen \( (\dot{V}E·\dot{V}O_2) > 30 \).

Samples of expired air were analysed for fractions of \( O_2 \) and \( CO_2 \) using a paramagnetic oxygen analyser (Servomex-Sybron/Taylor, model 570A) and a carbon dioxide analyser (Lira infra-red Gas analyser, model 303), and
the volume of expired air was measured by a Harvard dry gas meter. Expired air temperature was measured while evacuating the Douglas bags using a thermometer probe (Edale Instruments Ltd, model C). The barometric pressure was obtained from a barometer (Griffen and George Ltd). The Haldane transformation formula was used to calculate the inspired gas volumes, and all gas volumes were corrected to STPD. Oxygen uptake (\(\dot{V}O_2\)), carbon dioxide production (\(\dot{V}CO_2\)), minute ventilation (\(\dot{V}E\)), and respiratory exchange ratio (R) were calculated.

3.3.4.2. Blood lactate concentration during submaximal cycling

On a separate preliminary session (studies ii, iii, iv) subjects performed five continuous 4 min stages of submaximal cycling at 1Hz (60 rpm) at work rates corresponding to 40%, 50%, 60%, 70% and 80% of each individual's \(\dot{V}O_2\)max. Expired air was collected during the last minute of each stage and duplicate samples of arterialised capillary blood (20\(\mu\)l each) were taken from a pre-warmed thumb during the last 15 s of each stage for lactate determination. Heart rate was monitored throughout the test using short range telemetry (Sports Tester, Polar Electro Fitness, PE3000), and perceived rate of exertion was recorded every three minutes. The relationship between each individual's \(\dot{V}O_2\) and work rate during these submaximal intensities was always linear (r~0.998).

3.3.5. Standardised testing procedures

A standardised warm-up consisting of 4 min pedalling at 60 W (60 revs-min\(^{-1}\)) followed by 2x30 s at 80 and 100 W separated by 30 s of rest, preceded each test. The main tests consisted of cycle ergometer sprints against a resistance of 75 g·kg\(^{-1}\) body mass from a rolling start of approximately 1.2 Hz (70 revs-min\(^{-1}\)). Ten seconds before each sprint, the subject was instructed to start cycling at 70 revs-min\(^{-1}\) against a negligible resistance (loading basket lifted by experimenter), and then he was counted down into the sprint. Between "three" and "go" the loading basket was carefully and quickly lowered into position. On "go" the subject accelerated with maximal effort to maintain all-out sprinting. Subjects were instructed to avoid pacing. Strong verbal encouragement was given during each sprint. The importance of attaining maximal speed as soon as possible after the start of
each bout was emphasised. Sprints were separated by either active (Chapter VI) or passive recovery (Chapters IV, V, VII and VIII). Heart rate was recorded every minute throughout tests using short range telemetry (Sports Tester, Polar Electro Fitness, PE3000). Expired air samples were also collected in Douglas bags during sprints and during the recovery.

*Performance variables*

The following performance parameters were calculated for each sprint from the power output and pedal speed data:

- peak power output (PPO; attained 2 s into the sprint)
- pedal speed at which peak power was attained (SpPPO)
- maximum pedal speed (maxSp)
- mean power output for the first 6 s (MPO₆)
- mean power output for the first 10 s (MPO₁₀)
- mean power output for the last 20 s (MPO₂₀)
- mean power output for the whole sprint 30 s (MPO₃₀)
- mean pedal speed for the first 6 s (Sp₆)
- mean pedal speed for the first 10 s (Sp₁₀)
- mean pedal speed for the last 20 s (Sp₂₀)
- mean pedal speed for the whole sprint 30 s (Sp₃₀)
- the percentage decline from peak to end power output (fatigue index: FI)

\[
FI = \frac{\text{PPO-end power output}}{\text{PPO}} \times 100
\]

Additionally, the work done to accelerate the flywheel to peak speed (Wacc; usually during the first 3-5 seconds of the sprint) was calculated for each sprint as a measure of the ability of the subject to accelerate. The work done to accelerate the flywheel to maximal speed is the component usually ignored in calculations of power output during sprint cycling, resulting in underestimations of PPO of approximately 32% (Lakomy, 1986). Since this parameter takes into account the moment of inertia of the flywheel and its angular acceleration and velocity, it offers a good indication of the ability of the subject to accelerate the system.
3.4. COLLECTION, TREATMENT, STORAGE AND ANALYSIS OF BLOOD SAMPLES

3.4.1. SAMPLE COLLECTION

Venous blood samples were obtained from an antecubital vein using an indwelling cannula (venflon, 16-18 gauge) which was inserted under local anaesthetic (0.5 ml of 1% lignocaine). The cannula was inserted following 15-20 min of rest (in the seated position) on an examination couch, and a resting blood sample (8-10 ml) was obtained. The cannula was then flushed with sterile heparinised saline (10 U·ml\(^{-1}\)). Further blood samples were obtained at pre-determined times before and after sprinting and during recovery.

3.4.2. TREATMENT, STORAGE AND ANALYSIS OF BLOOD SAMPLES

Venous samples were placed in tubes containing lithium-heparin, and blood pH was measured immediately (Radiometer PHM73 pH/blood gas monitor). Duplicate (20 µl) aliquots were then taken from the venous blood for lactate determination. These samples and the capillary samples collected during the submaximal cycling test, were immediately deproteinised in 0.4 mol·l\(^{-1}\) (2.5%) of perchloric acid, stored at -20 °C and analysed at a later date using a fluorometric method (see APPENDIX A) described by Maughan (1982). The remaining blood (~1.5 ml) was placed in a Ca\(^{2+}\) heparinized tube (20 U·ml\(^{-1}\)), centrifuged at 13000 revs·min\(^{-1}\) (10400 g) for 3 min, and the supernatant was stored at -70 °C. Plasma ammonia was determined spectrophotometrically within 24 h of sampling\(^*\) (Boehringer Mannheim MPR 1; see APPENDIX A). Plasma sodium (Na\(^{+}\)) and potassium (K\(^{+}\)) concentrations were determined at a later date using flame photometry (Corning flame photometer, model 435).

\(^*\) A pilot study on the effect of freezing and storage conditions on plasma ammonia concentration conducted in this laboratory (Tsintzas and Wilson; unpublished observations) showed that plasma samples could be stored at -70 °C up to 48 hrs without any significant change in ammonia concentration. Moreover, there was no difference in ammonia concentration between samples snap-frozen in liquid nitrogen and samples stored at -70 °C.
Haemoglobin concentration was measured in duplicate 20 µl aliquots of venous blood using the Cyanmethemoglobin method (Boehringer Mannheim GmbH test-combination), and haematocrit was determined in triplicate after centrifugation in a microcentrifuge (Hawksley Ltd).

Changes in plasma volume were estimated from the changes in haemoglobin and haematocrit values (Dill and Costill, 1974). Due to the large effects of changes in body posture on plasma volume shifts (up to 10% decrease in plasma volume can occur when body posture changes from lying to standing; Rowell, 1993) all blood samples were taken while sitting on the couch or on the cycle ergometer. Only during study (i), all blood samples (except the immediately post sprint) were taken while the subjects were lying on a couch.

The coefficient of variation \( CV = \frac{\text{Standard Deviation}}{\text{mean}} \times 100 \) of the blood and plasma assays is shown in Table 3.1.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>n</th>
<th>Mean</th>
<th>±SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lactate</td>
<td>16</td>
<td>14.9</td>
<td>0.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Plasma sodium</td>
<td>20</td>
<td>139</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Plasma potassium</td>
<td>20</td>
<td>4.0</td>
<td>0.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Plasma ammonia</td>
<td>20</td>
<td>146</td>
<td>6</td>
<td>4.1</td>
</tr>
<tr>
<td>Blood pH</td>
<td>10</td>
<td>7.369</td>
<td>0.011</td>
<td>0.2</td>
</tr>
</tbody>
</table>
3.5. COLLECTION. TREATMENT. STORAGE AND ANALYSIS OF MUSCLE SAMPLES

3.5.1. MUSCLE BIOPSY PROCEDURE

Muscle biopsies were obtained from the middle portion of vastus lateralis using the percutaneous needle biopsy technique (Bergstrom, 1962) with suction being applied. The vastus lateralis muscle was selected because, apart from being easily accessible, it is free of major vessels and nerves, and is heavily involved during maximal cycling exercise (Bigland-Ritchie and Woods, 1974; Karlsson, 1971).

During the biopsy studies subjects reported to the laboratory at least 4 hours after a light meal and rested on a couch for 30 min. A cannula was placed in an antecubital vein, and small incisions through the skin and fascia over the vastus lateralis muscle of both legs were made under local anaesthesia (5 ml of 1% plain lignocaine). In half of the subjects the resting muscle biopsy was taken from the left leg, and subsequent biopsies were taken from alternate legs. The opposite order was used for the remaining subjects. All biopsies were obtained through different incisions in the skin. The time delay between cessation of the sprint and freezing of the sample in liquid nitrogen was on average between 5 and 7 s, and exact times are reported in each study.

Muscle samples were removed from the needle under liquid nitrogen (average size 30-100 mg wet weight), and stored in plastic screw-top tubes immersed in liquid nitrogen, until freeze dried (within 24 hours).

3.5.2. TREATMENT AND STORAGE OF MUSCLE SAMPLES

The freeze-dried muscle samples were washed twice with 1 ml petroleum ether to remove the fat and any blood present in the sample. Each time the sample was mixed using a whirlimixer (Fisons) before removing the ether. After the ether was removed for the second time, the tube was left open in a fume cupboard until the sample was completely dry.

Following that, the sample was dissected free of connective tissue and blood, and homogenised using an agate pestle and mortar. The powdered
samples were weighed using an electrical balance accurate to five decimal places, and stored at -70 °C in screw-top eppendorf tubes placed in bags with desiccant (silica gel).

3.5.3. ANALYTICAL METHODS.

3.5.3.1. Extraction procedure

At a later date, mixed muscle metabolites were extracted from the muscle powder as follows (Harris et al., 1974):

• Powdered muscle samples were removed from the freezer, and were allowed to warm at room temperature (~ 1 hour).

• Samples were centrifuged (SANYO microcentaur, MSE) for 3 min at 13000 revs·min⁻¹ (11300 g) to ensure that sample was at the bottom of the tube.

• The tip of each eppendorf tube was placed in liquid nitrogen and 100 μl of 0.5 mol·l⁻¹ HClO₄ with 1 mmol·l⁻¹ EDTA·Na₂ were added per mg dry muscle powder. Each sample was immediately placed in the freezer (-20 °C) until all samples were ready for agitation.

• Ten to twenty tightly sealed samples (one batch) were placed in a box with ice and agitated using a Stuart flask shaker for 30 min.

• Samples were then centrifuged for 10 min at 3 °C (Burkard Koolspin, μP refrigerated centrifuge) at 6000 revs·min⁻¹ (2400 g).

• The supernatant was then removed using a pipette, carefully measured (usually 10-20 μl less than the volume of HClO₄ originally added), placed into pre-cooled plastic tubes, and neutralised by adding 25 μl of 2.1 mol·l⁻¹ KHCO₃ per 100 μl of supernatant removed. The samples were then mixed and left on ice in the fridge (3 °C) with loose caps, to let the formed CO₂ escape.

• Samples were then centrifuged again for 5 min at 3 °C (6000 revs·min⁻¹ or 2400 g). Following this, the supernatant was removed, placed into new pre-cooled eppendorf tubes and kept on ice. The pH of the extract was 7.0 (neutralised).
3. 5. 3. 2. Mixed muscle metabolite assays

Assays were run and modified (see APPENDIX B and C) using extracts from horse, rat and human muscle until satisfactory results were obtained (see coefficients of variation, Table 3.2).

The muscle extracts from the main studies were analysed using modifications of the methods described by Harris et al., (1974) and Lowry and Passonneau (1972).

Phosphocreatine (PCr), creatine (Cr), ATP, ADP, AMP, free glucose, glucose-1-phosphate (G1P), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), pyruvate (Pyr), and lactate (La) were assayed enzymatically by fluorometric analysis (Locarte, model 8-9).

Glycogen was determined both on the neutralised extract (acid-soluble glycogen fraction), and on the muscle pellet left after the extraction procedure by prior HCl hydrolysis (acid-insoluble glycogen fraction), by measuring the glucosyl units obtained after acid hydrolysis of the glycogen (Jansson, 1981). Acid-insoluble glycogen was assayed spectrophotometrically (Cecil, CE 393) for glucose using a commercially available glucose kit (Boeringher Mannheim-Glucose, GOD/Perid method).

A detailed description of all muscle assays performed can be found in APPENDIX B.

Buffers, co-factors (Grade-I) and enzymes were obtained as standard commercial items from Boehringer and Sigma. All reagents were made up with double-distilled water the day before the analysis and kept at 4 °C. Standards were prepared on the day of the analysis using Grade-I chemicals. The molarity of the standards was checked on the same day spectrophotometrically using the coefficient of extinction of NADH/NADPH (APPENDIX C). The standard curves were always linear (r≥0.998). Metabolite concentrations (mmol·kg dry muscle⁻¹) were calculated using the resulting standard regression equation, taking into account double-distilled water blanks, extract blanks, standards and dilution factors. One ml of extract was equivalent to 8 mg of muscle powder. Where necessary, the concentrations of the metabolites were adjusted to the true molarity of the standards as calculated from the coefficient of extinction of NADH/NADPH.
All assays on the neutralised muscle extracts were performed within two days:

**DAY 1:** ATP, PCr and Cr were analysed immediately following the extraction procedure. ADP, AMP and Pyruvate were also assayed on that day. Extract was kept on ice and stored in the fridge between assays, and was frozen at -70 °C at the end of the day until the next day.

**DAY 2:** The free glucose, G1P, G6P, F6P, lactate and glycogen were assayed on day 2. The concentration of these metabolites has been shown to be unaffected by repeated freeze-thaw cycles of the muscle extracts.

Muscle metabolite contents were corrected to the individual mean total creatine content (PCr+Cr). The range of correction was between 0-11%. Since the sum PCr+Cr should not change during exercise, this acts as an internal reference in order to account for errors in muscle metabolite concentrations arising from the variable inclusion in the muscle samples of any remaining connective tissue, fat or blood (Harris et al., 1974). However, muscle lactate and glucose were not adjusted for total creatine content because the concentration of these metabolites in the blood (which is one of the contaminants in the muscle sample) is significant after heavy exercise (Harris et al., 1977; Marlin, 1989). Correction in this way may result in erroneously high values for muscle lactate. Correction for total creatine content has been shown to be a reliable method for normalising creatine phosphate and adenine nucleotides, and is comparable to methods like total protein or NAD⁺ content (Sabina et al., 1983). Normalisation to wet weight should be avoided, especially for post-exercise samples, due to the water shifts into the muscle (Harris et al., 1977; Sabina et al., 1983). All muscle metabolite concentrations are expressed as mmol·kg dry muscle⁻¹.

**3. 5. 3. Muscle pH determination**

**LIMITATIONS**
Measurement of muscle pH after homogenisation is a widely used, reliable and sensitive technique (Sahlin et al., 1975, 1976; Costill et al., 1982; Spriet et al., 1986; Marlin and Harris, 1991). However, the limitations of muscle homogenate pH measurements must be acknowledged:

- Muscle homogenate pH represents a mixture of extracellular and intracellular pH, and as such is influenced from the relative volume, pH and buffer capacity of each compartment present in the sample.
Homogenisation and measurement at 37 °C result in almost complete loss of ATP and PCr (Spriet et al., 1986; Marlin and Harris, 1991). However, phosphagen hydrolysis in resting and post electrical stimulation samples produced a slight, insignificant acidification and alkalinization of the homogenate, respectively (hydrolysis of ATP releases H⁺ and PCr breakdown consumes H⁺).

When homogenising wet muscle, CO₂ may be lost from the homogenate, resulting in an increase of pH. However Spriet et al. (1986) found that this is a slow process with a small influence on pH, which can be minimised by dilution of the sample.

Finally, the dilution of the muscle homogenate can influence muscle pH readings. Cheetham (1987) found that there was almost no effect of dilution when using 10-50 μl of homogenising solution per mg wet weight. However, Marlin and Harris (1991) reported that dilutions greater than 25 μl of homogenising solution per mg wet weight will result in underestimation of muscle pH.

**pH MEASUREMENT**

In this thesis muscle pH was determined after homogenization of freeze dried muscle powder (2-9 mg) at 4°C with an ice cold solution containing 145 mmol·l⁻¹ KCl, 10 mmol·l⁻¹ NaCl and 5 mmol·l⁻¹ iodoacetic acid (Sahlin et al., 1976; Costill et al., 1982). The iodoacetic acid (IAA) was included because it inactivates the enzyme glyceraldehyde-phosphate dehydrogenase, thereby inhibiting glycolysis at this step (Sahlin et al., 1976). The dilution ratio used was 100 μl of homogenizing solution per mg of dry muscle, which is the maximum dilution for reliable pH determinations (Marlin and Harris, 1991).

Samples were homogenised for 45 s in glass homogenising tubes using a motor driven homogeniser (Citenco Varicontrol, DTS 7333) at 3 increasing standardised speeds. Homogenates were then equilibrated to 37 °C for 5 min and pH was measured every 30 s using a MI-410 microelectrode (Microelectrodes, Inc.) connected to a Radiometer pH metre (Radiometer PHM73). Preliminary measurements showed that the temperature of 600-900 μl of homogenate reached = 37 °C after 2 min of equilibration. Therefore the average of all pH readings between 2 and 5 min of the equilibration period was taken as the muscle pH. Usually, the change in pH was small over the 5 min of equilibration.
The electrode was calibrated prior to each measurement using phosphate buffers, pH 6.841 and 7.383 (Radiometer, Copenhagen) and 6.0±0.02 and 6.5±0.02 at 37 °C. Calibration buffers were kept in a separate water bath (37 °C) to hasten the calibration procedure. After each measurement, the pH electrode was rinsed thoroughly with distilled water and cleaned with a deproteinising solution. The coefficient of variation for muscle pH determinations was 0.4% (Table 3.2).

Muscle pH determinations on freeze-dried muscle have an advantage over measurements using “wet muscle” because: (i) weighing of the sample is easier and more accurate, (ii) blood mixed with the muscle sample can be removed during dissection-powdering, and therefore contamination (usually seen as an increase in pH) is reduced (Harris et al., 1989). However, resting pH was found to be slightly higher in freeze dried, compared with wet muscle, and this was attributed to loss of bicarbonate in the form of CO₂ during freeze drying (Harris et al., 1989).

3.6. CALCULATIONS

The anaerobic ATP utilisation was calculated from the values of ATP, ADP, PCr, lactate and pyruvate before and immediately after the sprint, using the formula (Katz et al., 1986):

\[
\text{ATP utilisation} = 2(-\Delta\text{ATP}) - \Delta\text{ADP} - \Delta\text{PCr} + 1.5\Delta\text{La} + 1.5\Delta\text{Pyr}
\]

*2 active phosphates are cleaved per ATP utilised; 1.5 mmol ATP is produced for every mmol lactate and pyruvate.*

The mean anaerobic ATP utilisation rate (mmol·kg dry muscle⁻¹·s⁻¹) was obtained by dividing the anaerobic ATP utilisation by the duration of the sprint (10 s, 20 s or 30 s). In the case when ADP was not measured, the same formula was used but the change in ADP was omitted. Omission of ADP from the formula is not expected to make a significant difference, since total muscle ADP remains unchanged after sprint exercise (Nevill et al., 1989; Spriet et al., 1989), and this is the case even when ATP levels fall by 40-50% (Harris et al., 1991; Tullson and Terjung, 1991).

Glycogenolytic and glycolytic rates during the sprint (mmol glucosyl units·kg dry muscle⁻¹·s⁻¹) were calculated from accumulation of glycolytic metabolites as previously reported (Spriet et al., 1987):
glycogenolysis = $(\Delta G1P + \Delta G6P + \Delta F6P) + 0.5(\Delta La + \Delta Pyr)$

glycolysis = $0.5(\Delta La + \Delta Pyr)$

Since the exercise was dynamic and the circulation was not restricted by any mechanical means (e.g. tourniquet), some lactate diffused into the circulation during the sprints. However, due to the short duration of the exercise bout the underestimation in the above calculations is thought to be minimal.

Table 3.2. Coefficient of variation (CV, %) for muscle metabolite assays. Determinations are for $n=10$ (10 aliquots of extract from one sample) using human muscle samples (letter 'a'). Letter 'b' refers to 10 aliquots of powder from the same rat sample extracted separately. Values are mmol·kg dry muscle$^{-1}$ (except muscle pH).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>aMean ±aSD</th>
<th>aCV (%)</th>
<th>bMean ±bSD</th>
<th>bCV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI glycogen</td>
<td>260.3 ± 6.1</td>
<td>2.3</td>
<td>200.2 ± 7.1</td>
<td>3.1</td>
</tr>
<tr>
<td>AS glycogen</td>
<td>95.10 ± 3.90</td>
<td>4.1</td>
<td>42.87 ± 2.58</td>
<td>6.0</td>
</tr>
<tr>
<td>ATP</td>
<td>27.56 ± 0.70</td>
<td>2.5</td>
<td>25.78 ± 0.80</td>
<td>3.1</td>
</tr>
<tr>
<td>ADP</td>
<td>2.20 ± 0.10</td>
<td>4.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PCr</td>
<td>78.25 ± 1.90</td>
<td>2.4</td>
<td>25.05 ± 1.12</td>
<td>4.5</td>
</tr>
<tr>
<td>Cr</td>
<td>36.30 ± 1.01</td>
<td>2.8</td>
<td>80.9 ± 5.3</td>
<td>6.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.31 ± 0.20</td>
<td>3.8</td>
<td>2.43 ± 0.13</td>
<td>5.3</td>
</tr>
<tr>
<td>G1P</td>
<td>0.85 ± 0.04</td>
<td>4.7</td>
<td>1.12 ± 0.08</td>
<td>7.1</td>
</tr>
<tr>
<td>G6P</td>
<td>14.62 ± 0.48</td>
<td>3.3</td>
<td>9.85 ± 0.59</td>
<td>6.0</td>
</tr>
<tr>
<td>F6P</td>
<td>3.36 ± 0.10</td>
<td>3.0</td>
<td>2.35 ± 0.16</td>
<td>6.8</td>
</tr>
<tr>
<td>Lactate</td>
<td>30.10 ± 0.45</td>
<td>1.5</td>
<td>31.25 ± 0.70</td>
<td>2.2</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>2.09 ± 0.09</td>
<td>4.3</td>
<td>2.3 ± 0.09</td>
<td>3.9</td>
</tr>
<tr>
<td>Muscle pH$^+$</td>
<td>-</td>
<td>-</td>
<td>6.88 ± 0.25</td>
<td>0.4</td>
</tr>
</tbody>
</table>

AI glycogen, acid insoluble glycogen; AS glycogen, acid soluble glycogen; ATP, Adenosine triphosphate; ADP, Adenosine diphosphate; PCr, phosphocreatine; Cr, Creatine; G1P, Glucose-1-phosphate; G6P, Glucose-6-phosphate; F6P, Fructose-6-phosphate.

$^+$ muscle pH was determined on 10 homogenates of the same rat muscle sample.
The concentration of inorganic phosphate (Pi) in the muscle after the sprint and during recovery, was calculated from changes in ATP, ADP, PCr and hexose monophosphates (G1P, G6P, F6P), as in Bergstrom and Hultman (1988):

\[ \text{Pi} = 2.9 + \frac{2(-\Delta \text{ATP}) - \Delta \text{ADP} - \Delta \text{PCr} - (\Delta \text{G}1\text{P} + \Delta \text{G}6\text{P} + \Delta \text{F}6\text{P})}{3} \]

2.9 = resting value from Chasiotis (1983); 3l of intracellular water per kg dry muscle. Pi concentration is expressed in mmol·l⁻¹ muscle water.

In the case when ADP was not measured, the same formula was used but the change in ADP was omitted (see above). The percentage of Pi in the mono- and di-protonated forms (HPO₄²⁻ and H₂PO₄⁻) was calculated from muscle pH and the pK of Pi, using the Henderson-Hasselbalch equation (see APPENDIX E).

3.7. STATISTICAL ANALYSIS

One-way or two-way analyses of variance (ANOVA) for repeated measures on both factors were used where appropriate for statistical analysis. Where significant F ratios were found (P<0.05), the means were compared using a Tukey post-hoc test. Relationships between variables were examined by calculating the product moment correlation coefficient (r). Results are presented as mean ± standard error (SE).
CHAPTER IV

RECOVERY OF POWER OUTPUT AND MUSCLE METABOLITES FOLLOWING 30 s OF MAXIMAL SPRINT CYCLING

4.1. INTRODUCTION

The decline in force during maximal, short-term contractions has been associated with several metabolic changes in the exercising muscle such as a decrease in muscle phosphocreatine (Sjoholm et al., 1983; Katz et al., 1986), a corresponding increase in inorganic phosphate (Pi) and its diprotonated form, H$_2$PO$_4^-$, (Baker et al., 1992) and a marked fall in muscle pH (Cady et al., 1989). Although the accumulation of H$^+$, Pi and H$_2$PO$_4^-$ in the muscle cell may directly impair the activation of the contractile mechanism (Hermansen, 1981), other experiments have suggested that the decline in force may be related to the inability to regenerate ATP at the required rates (Sahlin and Ren, 1989; Soderlund et al., 1992).

Maximum rates of ATP regeneration are required during sprint exercise. The average rate of ATP regeneration from anaerobic sources during a 6 s sprint on a cycle ergometer is as high as 14.9 mmol·kg dry muscle$^{-1}$·s$^{-1}$ (Gaitanos et al., 1993), and a mean value for a 30 s sprint is ∼7.0 mmol·kg dry muscle$^{-1}$·s$^{-1}$ (Boobis et al., 1987). These high anaerobic ATP regeneration rates result in a 60-80% fall in phosphocreatine (PCr), a 30-40% fall in ATP and a several fold increase of glycolytic intermediates and lactate, as glycolysis supplies 65-70% of the anaerobic energy during a 30 s sprint (Cheetham et al., 1986; McCartney et al., 1986; Nevill et al., 1989).

Since PCr can regenerate ATP at very high rates, and its concentration in the muscle is limited, fatigue during short-term high intensity exercise may be related to PCr availability. However, the simultaneous drop in PCr and pH in this type of exercise, and the fact that PCr and H$^+$ are linked via the creatine kinase reaction (Sahlin et al., 1975), makes it difficult to separate their individual contributions to fatigue.
A very useful model for assessing the importance of the different muscle metabolite changes during high intensity exercise, is the study of the relationship between force or power output and metabolic state of the muscle during recovery from fatigue. During the initial recovery period, the relationship between PCr and [H+] disappears, allowing the study of their separate effects on power generation. A study by Harris et al. (1976) has shown that PCr resynthesis following exhaustive cycling (=9 min) or isometric exercise is a rapid process, with a half-time of ≈22 s. On the other hand, several studies using similar exercise modes have found that the half time for muscle lactate disappearance or pH restoration is in the region of 3-9.5 min (Sahlin et al., 1975, 1976). Interestingly, the pattern of peak force restoration after fatiguing isometric exercise is rapid and resembles that of PCr resynthesis (Baker et al., 1992; Sahlin and Ren, 1989). However, only a limited number of studies have followed power output recovery, which reflects variations in both force and velocity of contraction in the fatigued condition. The exercise intensities used in order to induce fatigue in these studies examining power output were either submaximal or ~120% $\dot{V}O_2$max and muscle metabolites and muscle pH were not measured (Sargeant and Dolan, 1987; Hitchcock, 1989).

The purpose of the present study was to follow PCr resynthesis, and the recovery of muscle pH, lactate and other muscle metabolites after maximal sprint exercise lasting 30 seconds. The relationship between PCr resynthesis, muscle acidosis and muscle function during the recovery period was also examined by parallel power output measurements which may provide more information about muscle function than isometric force restoration alone.
4.2. METHODS

4.2.1. Subjects

Fourteen male university students volunteered to participate in this study. Their mean (±SD) age, height and body mass were 23±2 years, 178±7 cm and 75.3±8 kg, respectively. All subjects were involved in regular training (athletics or games players; 5-6 times per week). Eight of the subjects participated in the second part of the study which involved muscle biopsies.

4.2.2. Experimental procedures and protocol

The modified friction-loaded cycle ergometer described in Chapter III interfaced with a microcomputer was used to record power output and flywheel speed (Lakomy, 1986). A restraining harness was passed around the subject's waist to restrict exercise to the leg muscles during the cycle ergometer sprints. The subjects were familiarised by completing at least two separate 1 hour sessions. Each subject performed all tests at the same time of day, which was at least four hours after any meal. Diet and exercise were controlled as previously described. The study consisted of two parts: performance of repeated 30s sprints and the muscle biopsy condition.

Repeateated sprints performance. All subjects (n=14) were required to perform two 30s maximal cycle ergometer sprints against a load of 75 g·kg⁻¹ body mass (average resistive load: 5.6±0.2 kg) from a rolling start of approximately 70 revs·min⁻¹, on three occasions. On each occasion the two sprints were separated by either 1.5 min, 3 min or 6 min of passive recovery on the bicycle seat (Fig. 4.1). Experimental tests were carried out in a random order, one week apart.

Peak power output (PP0), pedal speed at which peak power was attained (SpPP0), maximum pedal speed (maxSp), mean power output for the first 6 s, 10 s and the whole sprint (MPO6, MPO10 and MPO30), and the percentage decline from peak to end power output (fatigue index, FI) were calculated for each 30 s sprint. The work done to accelerate the flywheel to peak speed (during the first 3-4 s of the sprint; Wacc) was also calculated as a measure of the ability of the subject to accelerate.
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**Muscle biopsy condition.** After completion of the first part of the study, eight of the subjects agreed to have muscle biopsies taken. Subjects reported to the laboratory 4 hours after a light meal and rested on a couch for 30 min. A cannula was placed in an antecubital vein, and small incisions through the skin and fascia over the vastus lateralis muscle of both legs were made under local anaesthesia. Then subjects performed the standardised warm-up, and one maximal 30 s cycle ergometer sprint.

Needle-biopsy samples were obtained from the vastus lateralis muscle before the 30 s sprint, immediately after, and again after 1.5 min, 3 min and 6 min, while the subject was lying on a couch. In half of the subjects...
the resting muscle biopsy was taken from the left leg, and subsequent biopsies were taken from alternate legs. All biopsies were obtained through different incisions in the skin. The time delay between cessation of the sprint and freezing of the sample in liquid nitrogen was 7.5±1.6 s. Muscle samples were then removed from the needle under liquid nitrogen and freeze dried within 24 hours.

4.2.3. Analytical methods

Each freeze-dried sample was dissected free of connective tissue and blood and homogenised. Muscle powder was then divided in two parts. At a later date, one part of the powder was used to enzymatically determine muscle metabolites (Glycogen, PCr, Cr, ATP, ADP, AMP, free glucose, G1P, G6P, F6P, Pyr, and La) as described in Chapter III. Muscle metabolite contents (except lactate and glucose) were adjusted to the individual mean total creatine content (range of correction 0-11%). All muscle metabolite concentrations are expressed as mmol per kilogram dry muscle.

Muscle pH was determined on the second part of muscle powder using the homogenate technique (Chapter III).

4.2.4. Calculations

The mean rate of anaerobic ATP utilisation, glycolysis and glycogenolysis during the first 30 s sprint, as well as inorganic phosphate (Pi) and its diprotonated form were calculated as described in APPENDIX E.

The time course of PCr resynthesis during the recovery time (t), $PCr(t)$, was modelled for each subject separately, as described in APPENDIX F. Briefly, an exponential model with a power function exponent in time was fitted to each subjects' data. The general form of the model is:

$$PCr(t)=R-R\cdot\exp(-a\cdot t^b)$$  (1)

where $R$ denotes PCr at rest, and the parameters 'a' and 'b' (location and shape parameters) characterise each subjects' PCr resynthesis curve. For the present data a common group resynthesis model was shown to be inappropriate.
4.2.5. Blood sampling and analysis

During the repeated sprints performance (part 1 of study), duplicate samples of arterialised capillary blood (20μl each) were taken from a pre-warmed thumb for lactate determination at rest, after the standardised warm-up and on the 3rd and 5th minute after the second sprint in each condition (see Fig. 4.1). Capillary samples were also taken on the 3rd and 5th min after the first sprint when the recovery between sprints was 6 min.

Venous blood samples were obtained during the biopsy condition (part 2 of the study), at rest, and at the same time as the muscle biopsies (i.e. immediately after, 1.5, 3 and 6 min after the 30 s sprint; Fig. 4.1). Changes in plasma volume, blood pH and lactate, plasma ammonia and electrolytes (Na+, K+) were determined on the venous samples.

4.2.6. Statistical analysis

One-way or two-way analyses of variance (ANOVA) for repeated measures on both factors were used where appropriate for statistical analysis. Where significant F ratios were found (P<0.05), the means were compared using a Tukey post-hoc test. Relationships between variables were examined by calculating the product moment correlation coefficient (r). Results are presented as mean ± standard error (SE).
4.3. RESULTS

4.3.1. Power output during the first sprint

As this study had two parts, results will be reported for n=14 (repeated sprints performance) or n=8 (biopsy condition).

There were no significant differences in any of the power output indices of the first sprints between the three experimental conditions (1.5 min, 3 min and 6 min recovery intervals, n=14). Consequently, the mean values of the three first sprints will be presented. Values for n=8, who were subsequently biopsied are in parentheses.

The peak power output during the first sprint was 1264±55 W (1310±65 W), and was attained at a pedal speed of 148±3 (151±4) rpm. The highest pedal speed (maxSp) was attained 4 s after the start of the sprint, and averaged 160±4 (165±5) rpm, while the mean power output over the first 6 s and 30 s of the sprint was 931±33 W (954±39 W) and 647±18 W (660±24 W), respectively. The fatigue index was 65±2% (66±2%).

The work done to accelerate the flywheel to maximal speed (Wacc) is a parameter usually ignored in calculations of power output during sprint cycling, resulting in underestimations of PPO of approximately 32% (Lakomy, 1986). This parameter takes into account the moment of inertia of the flywheel and its angular acceleration and velocity and therefore offers a good indication of the ability of the subject to accelerate the system. The average Wacc during the first sprint was 839±46 J (888±57 J). It was found that the subjects with high Wacc also had a high PPO per kg body mass (r=0.96, P<0.01, n=14) and maxSp (r=0.97, P<0.01, n=14) during the first sprint.

High correlations were found in all three conditions between PPO and SpPPO during the first sprint (r=0.84 to 0.90, P<0.01, n=14), indicating that the more powerful subjects generated their PPO at higher pedal speeds. The subjects with the higher PPO during the first sprint also had the higher fatigue index in all conditions (r=0.84 to 0.88, P<0.01, n=14). The power output indices of the 30 s sprint performed during the biopsy condition (n=8) were not significantly different from the corresponding values during the first sprints of the repeated sprints sessions.
Peak power output was 1360±58 W and was generated at 152±3 rpm. The mean power output for 6 s and 30 s was 988±37 W and 664±24 W, respectively, and the FI 68±1%. A maximum pedal speed of 170±3 rpm was again attained on the 4th second of the sprint. Wacc averaged 971±45 J. Since the power output indices during sprint 1 were the same during all four occasions, and there was no order effect (ANOVA), the muscle metabolites measured during the biopsy condition were considered to be representative of all occasions.

4.3.2. Power output recovery

The power output and pedal speed profiles during sprint 1 (mean of the 3 conditions) and during sprint 2 after 1.5 min, 3 min and 6 min of passive recovery are shown in Fig. 4.2. Figure 4.3 shows the PPO, maxSp, MPO₆ and MPO₃₀, attained during the second sprint, after 1.5 min, 3 min and 6 min of recovery. Values are expressed as a percentage of the values attained during the corresponding sprint 1 (n=14). For maxSp and PPO, the zero time point represents the corresponding value at the last second of sprint 1 (52% and 35% of the peak values, respectively). There was no difference between the recovery curves for n=8 and n=14 (N.S.).

During the first 3 min of recovery, PPO and maxSp showed a rapid rate of restoration reaching 88.7% and 93.5% of sprint 1 values. However, no further increase in PPO and maxSp was observed during the remaining 3 min of recovery (Fig. 4.3). The recovery pattern for MPO₆ was similar to that of PPO and maxSp, but with a significant 3% increase between 3 and 6 min (P<0.01). In contrast, MPO₃₀ restoration followed a more linear pattern lacking the initial fast recovery. None of the sprint performance variables reached the control (sprint 1) values by the 6th min of recovery.

Strong negative correlations (r=-0.74 to -0.93; P<0.01, n=14) were found between PPO generated during the first sprint and %PPO, %SpPPO, %Wacc, %maxSp, %MPO₆ and %MPO₃₀ attained at the second sprint after 1.5 min and 3 min of recovery. These correlations were lower (r=-0.61 to -0.77; P<0.05 to P<0.01, n=14) during the 6 min recovery occasion. Similar relationships were obtained when the FI of the first sprint was correlated with all the above recovery variables, implying that subjects who fatigue more during sprint 1 (i.e. the more powerful subjects) have the lower rate of power output recovery.
Fig. 4.2. Power output (top) and pedal speed profiles (bottom) during sprint 1 (mean of the 3 conditions) and sprint 2 after 1.5 min, 3 min and 6 min of passive recovery.
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An interesting way to view the power output data during recovery is to divide the 30 s sprint into three 10 s parts. In doing so, the mean power for the first 10 s, the mean power for the middle 10 s and the mean power for the last 10 s of each 30 s sprint can be calculated. Fig. 4.4 illustrates this for sprint 1 (mean of the three conditions) and sprint 2 after 1.5 min, 3 min and 6 min of passive recovery. It is also apparent from Fig. 4.2 that power output during the last 10 s of sprint 2 was very similar between conditions, regardless of the recovery time. Similarly, small differences are seen in power output during the middle 10 s (10 s-20 s) of sprint 2 between conditions. As Fig. 4.4 shows, it is the mean power during the first 10 s of sprint 2 which recovers faster than the mean power during the rest of the
Fig. 4.4. Mean power output during the first 10 s (0-10 s), the middle 10 s (10-20 s) and the last 10 s (20-30 s) of sprint 1 and sprint 2 after 1.5 min, 3 min and 6 min of passive recovery. Top panel shows absolute values of mean power (W), while the lower panel shows mean power expressed as a percentage of the respective value during sprint 1. * P<0.01 from sprint 1; † P<0.01 from 1.5 min; § P<0.05 from 3 min; ‡ P<0.01 from 3 min; ** P<0.01 from mean power 10-20 s and 20-30 s.
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The faster recovery of the mean power output during the first 10 s of the sprint is a very important finding, since \( \approx 45\% \) of the total work during a 30 s sprint is done during the first 10 s. Therefore, a large part of the MP\(_{30}\) recovery depends on the recovery of power during the first 10 s of the sprint.

4.3.3. Muscle metabolites

The muscle metabolite concentrations at rest, immediately after the 30 s sprint, and 1.5 min, 3 min and 6 min into recovery are shown in Table 4.1. Muscle glycogen decreased by 35% (\( \approx 110 \) mmol glucosyl units·kg dry muscle\(^{-1}\)) and remained at that level during the rest of the recovery time. Interestingly, 85\% of the glycogen utilised during the 30 s sprint could be accounted for by the accumulation of the measured glycolytic intermediates, pyruvate (Pyr) and lactate (La). The PCr content of the muscle 7.5 s after the sprint was 19.7\( \pm \)1.0\% of the resting value, but PCr was rapidly resynthesised with a mean half-time of 56.6\( \pm \)7 seconds (Fig. 4.6). However, resynthesis of PCr was not complete after 6 min of recovery (85.5\( \pm \)3.5\% of the resting value). Predictions from the model gave an average time of 13.6 min (range 3.9-25 min) for PCr resynthesis to 95\% of the resting value. The large range of recovery times stresses the importance of considering the differences between individual PCr resynthesis curves (Fig. 4.5).

PPo, maxSp and MP\(_{06}\) restoration occurred in parallel with the resynthesis of PCr. In addition, there were high correlations between the individual %PCr resynthesis (relative to the resting value) and the percentage PPo, maxSp and MP\(_{06}\) attained during the second sprint, both after 1.5 min and 3 min of recovery (\( r = 0.71 \) to 0.86; \( P < 0.05 \)). An example from the correlations found is shown in Fig. 4.7. These correlations became lower and not significant for the 6 min recovery interval (%PCr-%PPo; \( r = 0.66 \), N.S.).

Muscle lactate increased to 119.0\( \pm \)4.6 mmol·kg dry muscle\(^{-1}\) immediately after the sprint, and subsequently decreased to \( \approx 90, 80 \) and 70\% of the peak value after 1.5 min, 3 min and 6 min of recovery (Fig. 4.6). The concomitant muscle acidosis, as quantified by muscle pH, is shown in Fig. 4.6. Muscle pH remained at the immediate post sprint levels for the first 3 min of recovery and then increased slightly to 6.79\( \pm \)0.02 after 6 min.
### Table 4.1. Muscle metabolites in vastus lateralis at rest, immediately after (POST), and 1.5 min, 3 min and 6 min after a 30 s cycle ergometer sprint.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>REST</th>
<th>POST</th>
<th>1.5 min</th>
<th>3 min</th>
<th>6 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (total)</td>
<td>321.5±18.2</td>
<td>211.6±18.5 a</td>
<td>223.2±19.5 a</td>
<td>217.2±21 a</td>
<td>221.0±18.3 a</td>
</tr>
<tr>
<td>acid insoluble</td>
<td>258.7±12.8</td>
<td>176.5±12.2 a</td>
<td>183.2±13.8 a</td>
<td>184.4±15.2 a</td>
<td>187.5±12.9 a</td>
</tr>
<tr>
<td>acid soluble</td>
<td>62.8±8.4</td>
<td>35.1±8.3 a</td>
<td>40.0±7.3 e</td>
<td>32.8±6.8 a</td>
<td>33.5±6.6 a</td>
</tr>
<tr>
<td>PCr</td>
<td>77.1±2.4</td>
<td>15.1±1.0 a</td>
<td>49.7±1.1 ab</td>
<td>57.2±2.0 abc</td>
<td>65.5±2.2 abcd</td>
</tr>
<tr>
<td>Cr</td>
<td>30.7±1.8</td>
<td>91.2±2.4 a</td>
<td>56.5±2.7 ab</td>
<td>48.6±2.9 abc</td>
<td>41.8±2.8 abcd</td>
</tr>
<tr>
<td>Total Creatine</td>
<td>107.8±3.2</td>
<td>106.3±2.5</td>
<td>106.2±2.9</td>
<td>105.8±2.1</td>
<td>107.3±2.7</td>
</tr>
<tr>
<td>Pi†</td>
<td>2.9</td>
<td>18.5±1.4 a</td>
<td>7.7±1.1 ab</td>
<td>7.4±1.3 ab</td>
<td>6.4±0.7 ab</td>
</tr>
<tr>
<td>H$_2$PO$_4^-$</td>
<td>1.09±0.02</td>
<td>10.4±0.96 a</td>
<td>4.82±0.75 eb</td>
<td>4.23±0.79 b</td>
<td>3.17±0.41 b</td>
</tr>
<tr>
<td>ATP</td>
<td>25.6±0.4</td>
<td>18.1±1.7 a</td>
<td>19.1±0.9 a</td>
<td>18.8±1.1 a</td>
<td>19.5±0.9 a</td>
</tr>
<tr>
<td>ADP</td>
<td>2.2±0.2</td>
<td>2.6±0.4 a</td>
<td>2.2±0.3</td>
<td>2.1±0.3</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>AMP</td>
<td>0.18±0.02</td>
<td>0.18±0.06</td>
<td>0.22±0.09</td>
<td>0.13±0.05</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.7±0.2</td>
<td>5.3±0.4 a</td>
<td>6.6±0.4 a</td>
<td>7.4±0.5 ab</td>
<td>7.8±0.6 ab</td>
</tr>
<tr>
<td>G1P</td>
<td>0.12±0.01</td>
<td>2.12±0.3 a</td>
<td>1.35±0.3 af</td>
<td>1.07±0.2 ab</td>
<td>0.84±0.2 ab</td>
</tr>
<tr>
<td>G6P</td>
<td>1.21±0.2</td>
<td>22.8±1.2 a</td>
<td>20.9±0.6 a</td>
<td>16.6±0.8 abc</td>
<td>11.0±1.2 abcd</td>
</tr>
<tr>
<td>F6P</td>
<td>0.13±0.02</td>
<td>6.4±0.3 a</td>
<td>5.2±0.2 ab</td>
<td>4.0±0.3 abc</td>
<td>2.6±0.3 abcd</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.95±0.1</td>
<td>4.6±0.4 a</td>
<td>1.9±0.2 eb</td>
<td>1.6±0.1 b</td>
<td>1.5±0.1 b</td>
</tr>
<tr>
<td>Lactate</td>
<td>3.8±0.3</td>
<td>119.0±4.6 a</td>
<td>107.3±3.8 a</td>
<td>95.4±5.6 ab</td>
<td>81.9±6.0 abcg</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 subjects, expressed in mmol·kg dry muscle$^{-1}$. Muscle glycogen expressed in mmol glucosyl units·kg dry muscle$^{-1}$; † Pi and H$_2$PO$_4^-$, calculated total and diprotonated form of inorganic phosphate (mmol·l muscle water$^{-1}$). Significant differences:

- a = $P<0.01$ from REST;
- b = $P<0.01$ from POST;
- c = $P<0.01$ from 1.5 min;
- d = $P<0.01$ from 3 min;
- e = $P<0.05$ from REST;
- f = $P<0.05$ from POST;
- g = $P<0.05$ from 3 min.
A high correlation was found between the muscle pH and La+Pyr contents after the sprint ($r = -0.94; P<0.01$). Since muscle pH remained depressed and [La] was high during recovery, they were both unrelated to the percentage sprint performance restoration ($P>0.05$).

Muscle ATP was decreased by ~ 30% after the sprint, and was not restored during recovery (Table 4.1). Muscle glucose was elevated at the end of the sprint and continued to increase until the end of the observation period. The concentrations of G1P, G6P and F6P increased 18fold, 19fold and 49fold, respectively, and were still elevated after 6 min of recovery. The subjects with the higher MPO per kg body mass had the highest post sprint [G6P] ($r = 0.84, P<0.01$).

Calculated Pi concentration increased considerably after the sprint (Table 4.1). After 1.5 min of recovery Pi was decreased to 7.7 mmol·l$^{-1}$ muscle water and thereafter remained unchanged until the end of the recovery period. Calculated [$H_2PO_4^-$] followed a similar pattern remaining unchanged between 1.5 and 6 min of recovery.

![PCr resynthesis curves](image)

**Fig. 4.5.** Individual PCr resynthesis curves fitted using the model described in APPENDIX F. Values for PCr are expressed as a percentage of the resting concentration. Numbers 1-8 represent individual subjects.
Fig. 4.6. Time course of phosphocreatine (PCr) resynthesis and muscle lactate disappearance (top) and muscle pH changes (bottom) during recovery from a 30 s maximal cycle ergometer sprint. Values for PCr are expressed as a percentage of the resting concentration and for lactate as a percentage of the peak content (mean±SE, n=8). The curve fitted on the PCr data represents the mean of the curves fitted on the individual data for each subject. * P<0.01 from REST. † P<0.01 from 1.5 min. Due to lack of biopsy material, pH was determined for n=6 for the POST and 6 min sampling points, and n=7 for the 1.5 min point. The resting and 3 min pH determinations were for n=8.
4. 3. 4. ATP utilisation and glycogenolytic and glycolytic rates

The mean ATP utilisation rate was 8.5±0.9 mmol·kg dry muscle⁻¹·s⁻¹ (total ATP utilisation was 255±10 mmol·kg dry muscle⁻¹), of which 69.9±1% was supplied from glycolysis, and 24.4±1% from PCr. The ATP utilisation rate was closely related to the glycogenolytic rate (r = 0.87, P<0.01). The glycogenolytic and glycolytic rates during the 30 s sprint were 3.0±0.1 and 2.0±0.1 mmol glucosyl units·kg dry muscle⁻¹·s⁻¹, resulting in a glycogenolysis to glycolysis ratio of 1.5±0.02. This was a consequence of a large build-up of hexose monophosphates (G1P, G6P, F6P).
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4.3.5. Blood lactate and blood pH

The standardised warm-up did not result in a significant increase of blood lactate concentration ([BL]; rest: 0.7±0.1 vs post warm up: 1.1±0.1 mmol·l⁻¹; N.S.). The peak lactate values, observed 5 min after the second 30 s sprint (repeated sprint performance), were similar in the 1.5 min and 3 min recovery conditions (16.9±0.5 and 16.6±0.4 mmol·l⁻¹), but a higher [BL] was seen in the 6 min recovery condition (17.4±0.4 mmol·l⁻¹, P<0.01; see Fig. 4.9).

The blood lactate responses (venous blood) to one 30 s cycle ergometer sprint were examined during the biopsy condition (Fig. 4.9). The highest [BL] was observed 6 min after completion of the sprint, and was 13.6±0.9 mmol·l⁻¹. The blood pH was also measured on this occasion (Fig. 4.10). Blood pH dropped from a resting value of 7.38±0.01 to 7.08±0.03, and remained depressed until the end of the observation period. The subjects with the higher PPO and MPO₃₀ also had the higher [BL] on the 6th min of recovery (r=0.93; P<0.01). Blood lactate and pH were inversely correlated throughout recovery (r= -0.95 to -0.96, P<0.01). There was no relationship between blood and muscle lactate or blood and muscle pH for corresponding time samples throughout recovery (r<0.35; N.S.).
Fig. 4.9. Blood lactate concentration at rest, after warm-up and 3 min and 5 min after sprint 2 (S2) during the three experimental conditions (top). The lower panel shows blood lactate concentration during the biopsy condition when only one 30 s sprint (2) was performed (n=8). * P<0.01 from rest; ‡ and † P<0.01 and P<0.05 from 1.5 min; § P<0.01 from 3 min; # P<0.01 from POST.
4. 3. 6. Plasma ammonia

Resting plasma ammonia concentration was 29.1±4.7 μmol·l^{-1} and was elevated to 94.0±17.6 μmol·l^{-1} after the 30 s sprint. The plasma ammonia concentration increased throughout recovery, reaching 156.9±20.0 μmol·l^{-1} after 6 min (Fig. 4.10). The individuals with the higher PPO and MPO30 also had the higher peak plasma ammonia concentration (r= 0.85 and 0.86, P<0.01). Strong correlations were found between blood lactate and pH, and ammonia concentration throughout recovery (r= 0.84 to 0.90, P<0.01, ammonia and lactate; and r= -0.80 to -0.86, P<0.05 to 0.01, ammonia and pH).

![Fig. 4.10. Plasma ammonia and blood pH at rest and during recovery from a 30 s maximal cycle ergometer sprint (mean±SE, n=8). * P<0.01 from rest. † P<0.01 from POST; ‡ P<0.01 from 1.5 min.](image)

4. 3. 7. Plasma electrolytes, changes in plasma volume and heart rate

Venous plasma sodium ([Na^{+}]) and potassium ([K^{+}]) concentrations before and during recovery from one 30 s sprint are shown in Fig. 4.11. Although [Na^{+}] increased by an absolute value of ~6 mmol·l^{-1}, the largest relative increase was in [K^{+}] (45% above resting value). If the measured electrolyte concentrations are corrected for changes in plasma volume, then a decrease (~17 mmol·l^{-1}) of Na^{+} content of the blood is seen, due to the expected loss of Na^{+} from the circulation. The [K^{+}] recovery pattern was,
nevertheless, little affected by changes in plasma volume. At the end of the recovery period of 6 min [K⁺] had dropped below resting values.

![Graph showing plasma K⁺ and Na⁺ concentrations](image)

**Fig. 4.11. Plasma K⁺ (top) and Na⁺ concentrations (bottom) before and during recovery from a 30 s sprint. Dashed line on top graph indicates resting concentration. Values are mean±SE, n=8. ** P<0.01 from rest; * P<0.05 from rest; † P<0.01 from POST; ‡ P<0.01 from 1.5 min; # P<0.05 from 1.5 min; § P<0.01 from 3 min; 2 30 s sprint.**

The average decrease in plasma volume immediately after the sprint was 15.9±2.2% and recovered very little (11.7±2.7%, N.S.), 6 min into recovery.
Peak heart rate during sprint 1 was 173±4 beats·min⁻¹ and similar values were observed during sprint 2 in all conditions (175±2, 174±3, and 171±3 after 1.5 min, 3 min and 6 min of recovery).
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4.4. DISCUSSION

This study examined muscle metabolism and performance restoration after a maximal 30 s cycle ergometer sprint. The main findings were that the resynthesis of PCr and the restoration of peak performance (PPO, maxSp, MPO6) proceeded in parallel, even though muscle pH remained low (~6.7) throughout the recovery. In addition, PCr resynthesis was slower than that previously observed after dynamic exercise of longer duration (Harris et al., 1976). These observations allow some comments to be made on the relative importance of energy supply and the direct effect of H\(^+\) on the contractile mechanism in the aetiology of fatigue during sprinting.

4.4.1. Power output recovery

During the first few seconds of a maximal sprint, energy is made available through the rapid degradation of PCr and anaerobic glycolysis. For example, during a 6 s sprint on a cycle ergometer the mean rate of PCr degradation averaged 7.3 mmol·kg dry muscle\(^{-1}\)·s\(^{-1}\) and PCr contributed approximately 50% to the total anaerobic ATP production (Gaitanos et al., 1993). However, if exercise is continued for 30 s, as in the present study, PCr will be so low after 10 s (Jones et al., 1985) that ATP production during the remaining 20 s will be dependent predominantly on the glycolytic rate and aerobic metabolism. Thus, if energy supply is more critical to power generation than the direct effect of H\(^+\) on the contractile mechanism, then performance during the initial seconds of the second sprint will reflect PCr resynthesis and any reduction in the glycolytic rate, rather than the recovery in muscle pH. Furthermore, peak and mean (MPO30) power output restoration may be differently affected as a result of the varying contributions of PCr and glycolysis to energy supply in the first few seconds and over the entire 30 s of the sprint.

In the present study the similar patterns of, and the statistically significant correlations between, PCr and peak performance restoration support the idea that PCr availability is critical for power generation during the initial seconds of the sprint. A possibility exists that PCr resynthesis reflects the removal of inorganic phosphate and its acidic fraction (H\(_2\)PO\(_4\)) which may be related to power recovery, rather than PCr availability per se. However, the calculated Pi and its acidic fraction, H\(_2\)PO\(_4\) (Table 4.1) did not change
between 1.5 and 6 min after the sprint, while power and PCr recovered significantly.

Furthermore, it is possible that changes in PCr may account for changes in power output in early recovery as a result of similar inhibition of glycolysis at 1.5 and 3 min, due to the lack of change in muscle pH. While the interaction of the variables controlling the rate of glycolysis are not fully understood, it has been reported that the glycolytic rate is reduced by 40-60% when muscle pH is decreased to ~6.7 (Spriet et al., 1987b, 1989).

Thus, the initial slower restoration of mean power output (MPO30) in comparison with peak power output may be explained by the continued inhibition of glycolysis, while PCr is rapidly resynthesized. At this point it must be noted that a significant part of MPO30 recovery can be attributed to the recovery of the mean power during the initial seconds of the second sprint (and therefore PCr resynthesis), since 45% of the total work done during the sprint is generated during the initial 10 s (see Fig. 4.4). After 6 min of recovery muscle pH increased, and the inhibiting effect on the phosphofructokinase activity (Trivedi and Danforth, 1966; Spriet et al., 1987b) was possibly less, allowing more ATP to be regenerated through glycolysis and further recovery of MPO30.

The suggestions that energy supply and particularly PCr availability are critical for short-term power production are supported by recent studies which have shown that oral creatine supplementation increased muscle PCr content (Harris et al., 1992) and enhanced performance during repeated bouts of high-intensity exercise (Greenhaff et al., 1993; Balsom et al., 1993a; Greenhaff et al., 1994c).

Although PCr and power output recovered at an initial rapid rate in the present study, 6 min of rest following a maximal 30 s sprint were inadequate for muscle metabolism and power output to recover fully. The incomplete recovery of all power output indices may be related to the low pH which impairs glycolysis, and also to the PCr which after 6 min was only ~85% of the resting value. Support for the notion that the effect of [H+] on the ATP generating process (i.e. glycolysis) is more important than an effect on the contractile mechanism itself, is given by a study which followed the recovery of isometric force after a fatiguing static contraction.
CHAPTER IV: Muscle metabolism and power recovery after sprinting

(Sahlin and Ren, 1989). Maximum voluntary force (MVC) was back to the pre-fatigue value 2 min after isometric knee extension to exhaustion (~52 s duration) at 66% MVC, despite high muscle lactate. Furthermore, in the present study some subjects exhibited almost full recovery (96-98%) of PPO, maxSp and MPO6 values after 3 min of rest, which was matched by a high PCr resynthesis despite a pH of ~6.79.

A possible explanation for the apparent plateau in PPO and maxSp recovery may be given by selective fatigue of fast twitch fibres. During all-out sprint cycling exercise, fast twitch motor units are important for high power output generation (McCartney et al., 1983). However, due to the higher glycogenolytic and PCr degradation rates in the fast twitch (FT) compared with the slow twitch (ST) fibres (Greenhaff et al., 1994), the FT fibres may accumulate more H+ and be subject to an 'energy crisis' which will result in lower force and therefore power generation (Soderlund et al., 1992). Moreover, it has been reported that PCr resynthesis is slower in the FT fibres (Soderlund and Hultman, 1991; Tesch et al., 1989) possibly due to higher [H+] and a poorer capillary network supplying the FT fibres (Tesch and Wright, 1983). Therefore an impaired ability of FT fibres to regenerate ATP at high rates, due to slower PCr resynthesis in these fibres, may explain the levelling-off of PPO and maxSp recovery observed in the present study after the 3rd min of rest. The finding that the subjects with the higher PPO during the first sprint had the lower power recovery may be related to the above hypotheses, since subjects with high PPO usually have a high percentage of FT fibres (McCartney et al., 1983).

Comparison of the results of the present study with the few studies examining power recovery, reveals that the recovery of power output may be related to the duration and intensity of prior exercise: shorter duration and lower intensity of previous exercise, is associated with a faster recovery of muscle function. In the study of Sargeant and Dolan (1987) maximal peak power reached the control values after only 1 min of recovery. The previous exercise was cycling for 6 min at an intensity equivalent to 87% VO2max. Similarly, Hitchcock (1989) reported full recovery of power output 1 min after a 2 min cycling bout at 60% and 80% VO2max. However, power remained at ~87% of the initial value for 4 min after cycling at 120% VO2max for 1.5-2 min (Hitchcock, 1989). In the present study, the average intensity is about 2.5 times greater than that which
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would elicit the subjects' $V_{O2}\text{max}$. Furthermore, the high energy demand during sprint exercise results in a mismatch between energy production and utilisation, and the magnitude of the metabolic stress is reflected by low muscle pH and 30% decrease in ATP, which is accompanied by an increase in AMP deamination and a corresponding increase of plasma ammonia concentration (Sahlin & Katz, 1988; Harris et al., 1991), as observed in the present study. Therefore the magnitude of the metabolic disturbances may explain the slower power recovery in the present study.

4.4.2. PCr recovery

In addition to the slower power recovery, PCr resynthesis was also slower than that previously observed after cycling exercise at 60 rev.min$^{-1}$ to exhaustion lasting $\approx 8.7$ min (Harris et al., 1976). From the model of PCr resynthesis, it can be calculated that PCr was restored at an initial average rate of 2.4 mmol ·kg dry muscle$^{-1}$·s$^{-1}$. This initial resynthesis rate value was similar to that observed by Harris et al. (1976), but the half-time of PCr resynthesis was much longer in the present study (56.6±7.3 s vs 22 s). In earlier studies it has been postulated that the initial fast phase of PCr resynthesis is an oxygen dependent process, linked with oxidative phosphorylation and the mitochondrial creatine kinase activity, while the subsequent slow phase is limited by the recovery of muscle pH because of the effect of H$^+$ on creatine kinase equilibrium (Sahlin et al., 1975, 1979). The common regulator for oxygen availability and clearance of H$^+$ from the muscle is blood flow. The slow resynthesis of PCr and pH recovery in the present study may have been due to the passive recovery between sprints, resulting in pooling of blood in the legs thereby reducing blood flow, H$^+$ clearance and O$_2$ supply (Spriet et al., 1989). The importance of blood flow in the recovery of muscle metabolites has been previously demonstrated by occluding the circulation immediately after exercise, which completely prevented PCr resynthesis, pH restoration and power output recovery (Harris et al., 1976; Sahlin et al., 1979; Gaitanos, 1990).

The large variation in PCr resynthesis between subjects in the present study (Fig. 4.5) stresses the importance of following the individualised modelling approach described here, as opposed to a common curve fit for all the subjects. The variability among subjects may be related to differences in muscle fibre composition (Soderlund and Hultman, 1991; Tesch et al., 1989) and training status since an increased capillary density,
which accompanies endurance training, is related to a faster recovery of isokinetic knee extension torque following fatiguing contractions (Tesch and Wright, 1983).

4.4.3. Blood metabolites

A common observation regarding changes in blood metabolites after sprint exercise is an increase in plasma ammonia (e.g. Snow et al., 1992). An increase in plasma ammonia during this type of exercise may be a reflection of adenine nucleotide loss from the exercise muscle as AMP is deaminated to IMP and ammonia (Sahlin and Katz, 1988; Hellsten-Westing et al., 1993). In the present study peak plasma ammonia concentration was highly correlated with peak and mean power output and blood lactate and pH, implying that the most powerful individuals (who exhibit the larger metabolic changes) are losing more ATP as a consequence of the high exercise intensity. In support of this argument there was a tendency for the post sprint ATP concentration to be associated with peak plasma ammonia (r=-0.64, N.S.), a result frequently reported by others following intense exercise in the horse (Harris et al., 1991; Sewell et al., 1992). The loss of adenine nucleotides from the muscle probably reflects an "energy crisis" and is associated with transient increases of ADP and AMP when PCr drops to very low levels (Funk et al., 1989). Muscle acidosis also stimulates AMP deaminase (Dudley and Terjung, 1985) but it is not a prerequisite for AMP deamination since McArdle's patients lose ATP and rapidly accumulate ammonia despite no H⁺ production due to lack of phosphorylase (Sahlin et al., 1990). The finding that plasma ammonia is related to power output in the present study is in accordance with Dudley et al. (1983) who have shown that individuals with a high proportion of fast twitch fibres have a higher blood ammonia after intense exercise.

The rapid increase in plasma potassium seen immediately after the 30 s sprint in the present study, has been previously observed after isokinetic cycling (Kowalchuk et al., 1988a) and has been attributed to an insufficient stimulation of the Na⁺-K⁺ pump of the exercising muscle (Medbo and Sejersted, 1990). Similarly, the drop of plasma K⁺ below the resting values during the recovery period has been explained by a higher gain of the pump after exercise due to the high extracellular potassium (Medbo and Sejersted, 1990). Potassium fluxes from and to the muscle have been
identifying as a factor contributing to fatigue through transmission failure of the action potential in the T-tubules (Sjogaard, 1990). However, the Na\(^+\) - K\(^+\) pump is energy dependent and a fall in the "local" membrane ATP may be critical for its function (Sjogaard, 1990).

In summary, this study demonstrated that the restoration of peak power output parameters (PPO, maxSp, MPO\(_6\)) during recovery from a 30 s bout of maximal sprint exercise occurred in parallel with PCR resynthesis, in spite of the low muscle pH. However, neither PCR nor sprint performance recovery were complete after 6 minutes of rest. The slow PCR resynthesis after this type of exercise may be related to the high exercise intensity and to a reduced blood flow in the legs during recovery. From the results of this study, it seems that the inability to regenerate ATP at high rates from PCR and glycolysis is related to the decreased power output during recovery from maximal sprint exercise.
CHAPTER V

POWER OUTPUT AND ENERGY SUPPLY DURING REPEATED SPRINT EXERCISE

5.1. INTRODUCTION

In the previous study it was suggested that PCr plays an important role in the recovery of power output during the initial seconds of a repeated 30 s sprint. This was based on the parallel time course of PCr resynthesis and peak power output parameters, in spite of the low muscle pH which remained depressed throughout recovery. However, muscle metabolism during the second sprint was not examined in that study and relatively little information exists regarding the relationship between muscle metabolism and power output during repeated sprint exercise.

Phosphocreatine is a high energy phosphate store which can rephosphorylate ADP and therefore regenerate ATP at rapid rates (Sahlin, 1986a). Its faster restoration after an intense exercise bout compared with other metabolites (Harris et al., 1976; Sahlin et al., 1976), makes PCr a readily available energy store. However, during recovery from a 30 s sprint, PCr is not fully resynthesised even after 6 min of rest (Chapter IV). This would suggest that its contribution to energy supply will be decreased during a second sprint. Furthermore, data from studies using electrical stimulation (Hultman et al., 1990; Soderlund et al., 1992) or sprint cycling for a period of 5-10 s have shown that PCr breakdown is a very rapid process which may be as fast as 7.3 mmol·kg dry muscle$^{-1}$·s$^{-1}$ (Gaitanos et al., 1993). This would imply that the whole PCr store may be used during the first few seconds of the second sprint, leaving the muscle to rely on the glycolytic and aerobic energy supply.

Although it has been suggested that low muscle pH may inhibit glycogenolysis and glycolysis when 30 s sprints are repeated (McCarterney et al., 1986; Spriet et al., 1989), no study has directly compared these changes from sprint 1 to sprint 2 and their effect on power output. In the study of Spriet et al. (1989) muscle biopsies were obtained before and after the
second 30 s sprint, but not before and after the first one which was performed 4 min earlier. Muscle glycogen breakdown during sprint 2 was \( \approx 47 \text{ mmol·kg dry muscle}^{-1} \). Surprisingly, only 51% of this glycogen could be accounted for as pyruvate, lactate or glycolytic intermediates in the muscle. This discrepancy may be partially explained by an efflux of lactate to the blood during the sprint, but it may also be that part of that glycogen was utilised aerobically. A significant contribution of aerobic metabolism to energy supply during short term-intense exercise has been suggested by other authors (McCartney et al., 1986; Medbo and Tabata, 1989). It seems therefore that the decrease in glycolytic energy supply when sprint exercise is repeated may be partially compensated by an increase in aerobic metabolism.

The above evidence would suggest that all the main energy systems (PCr, anaerobic glycolysis and aerobic metabolism) may contribute significantly to energy supply during a repeated 30 s sprint. However, it is possible that PCr may be utilised during the initial 10 s of the second sprint, and it is tempting to speculate that if the rate of lactate production is initially high, the concomitant decrease in muscle pH will significantly reduce glycolysis and glycogenolysis after the first 10 s of sprint 2.

Therefore, the purpose of the present study was to examine muscle metabolism during the first 10 s and the last 20 s of a second 30 s sprint. By doing so, the contribution of PCr and glycolysis at the start, and towards the end of sprint 2 will be assessed. Furthermore, the relationship between power output recovery and changes in muscle metabolites will be examined.
5.2. METHODS

5.2.1. Subjects

Eight male university students aged 24±2 years (mean±SD) height 177±7 cm and body mass 79±10 kg, volunteered to take part in this study. All subjects were physically active (recreational athletes).

5.2.2. Experimental procedures and protocol

A modified friction-loaded cycle ergometer (Chapter III) interfaced with a microcomputer was used to record power output and flywheel speed (Lakomy, 1986). A restraining harness was passed around the subject's waist to restrict exercise to the leg muscles during the cycle ergometer sprints. The subjects were familiarised with sprint cycling by completing at least 3-4 separate sprint practice sessions.

5.2.2.1. Preliminary tests

During a preliminary visit, the maximum oxygen uptake (V̇O₂_max) of each subject was determined using a continuous incremental test on the Monark cycle ergometer (Chapter III). On a separate session, subjects performed five continuous 4 min stages of submaximal cycling at work rates corresponding to 61±2%, 71±2%, 80±2%, 86±2% and 93±2% V̇O₂_max. Expired air was collected during the last minute of each stage and duplicate samples of arterialised capillary blood (20μl each) were taken from a pre-warmed thumb during the last 15 s of each stage for lactate determination. From this test, the relative intensity (%V̇O₂_max) corresponding to a blood lactate concentration of 4 mmol·l⁻¹ was determined for each subject by linear interpolation.

5.2.2.2. Main tests

Subjects were required to perform two maximal cycle ergometer sprints, separated by 4 min of passive recovery on the bicycle seat, on two randomly assigned occasions, one week apart. On one occasion a 30 s sprint was followed by another 30 s sprint (30-30 condition) and on the other occasion a 30 s sprint was followed by a 10 s sprint (30-10 condition; Fig. 5.1). Each subject performed all tests at the same time of day, which was at least four hours after any meal. Diet and exercise were controlled
CHAPTER V: Muscle metabolism during repeated sprint exercise

for 2 days prior to each test (Chapter III). The resistive load was 75 g·kg⁻¹ body mass (average load: 5.9±0.3 kg) and each sprint started from a rolling start of approximately 70 rev·min⁻¹.

The following performance parameters were obtained for each sprint: Peak power output (PP0), pedal speed at which peak power was attained (SpPP0), maximum pedal speed (maxSp), mean power output for the first 10 s (MPO10), the last 20 s (MPO120) and the whole sprint (MPO30), and the percentage decline from peak to end power output (fatigue index, FI). The mean pedal speed during the above time intervals was also calculated (Sp10, Sp120, Sp30). The work done to accelerate the flywheel to peak speed (during the first 3-4 s of the sprint; Wacc) was calculated as a measure of the ability of the subject to accelerate.

On arrival at the laboratory subjects rested on a couch (sitting position) for 30 min, while a cannula was placed in an antecubital vein and small incisions were made through the skin and fascia over the vastus lateralis muscle of both legs, under local anaesthesia. A resting blood sample was then obtained and the resting biopsy was taken. Further biopsies and blood samples were taken immediately after the first sprint and before and after the second sprint while the subject was sitting on the cycle ergometer (Fig. 5.1). A total of five biopsies were taken from each subject through different incisions in the skin: at rest, post sprint 1, before sprint 2 (3.8±0.01 min after the end of sprint 1), after the second 10 s sprint and after the second 30 s sprint. The biopsy leg and testing order were randomised in a balanced design, so that biopsies before and immediately after each sprint were taken from the same leg. The time delay between cessation of the sprint and freezing of the biopsy sample in liquid nitrogen was 6.6±0.4 s for sprint 1, 5.3±0.5 s when sprint 2 was 10 s and 6.2±0.6 s when sprint 2 was 30 s. Muscle samples were kept in liquid nitrogen until they were freeze dried (within 24 hours of sampling).

Expired air samples were collected in Douglas bags during each sprint and heart rate was recorded using short range telemetry throughout the test. Expired air samples were analysed as previously described.
Fig. 5.1. Schematic representation of the experimental design. A total of 5 muscle biopsies (↑) were obtained from each subject during the two conditions. ♦, venous blood samples; □, 30 s maximal sprint; □, 10 s maximal sprint.

5.2.3. Analytical methods

The freeze dried samples were dissected free of connective tissue and blood and homogenised. Muscle powder was then divided in two parts. At a later date, one part of the powder was used to enzymatically determine muscle metabolites (Glycogen, PCr, Cr, ATP, free glucose, GIP, G6P, F6P, Pyr, and La) as described in Chapter III. Muscle metabolite contents (except lactate and glucose) were adjusted to the individual mean total creatine content. All muscle metabolite concentrations are expressed as mmol·kg dry muscle⁻¹. Muscle pH was determined on the second part of muscle powder using the homogenate technique (Chapter III).

The mean rate of anaerobic ATP utilisation, glycolysis and glycogenolysis during each sprint, as well as inorganic phosphate (Pi) and its diprotonated form were calculated as described in APPENDIX E.

Venous blood samples were analysed for blood lactate, plasma ammonia and plasma electrolytes (Na⁺, K⁺). Blood pH was measured immediately after the sample was drawn and changes in plasma volume were calculated from changes in haematocrit and haemoglobin (Dill and Costill, 1974).
The contribution of aerobic metabolism to energy supply during each sprint was estimated from the \( \dot{V}O_2 \) measurements and the following assumptions:

(a) Working muscle mass was calculated as 20% of body weight (Wootton, 1984; Jones and Pearson, 1969).

(b) The conversion of wet to dry muscle weight was done assuming that muscle water content at rest was 76.5% of the total weight (Kowalchuk et al., 1988; Bangsbo et al., 1992).

(c) The \( P/O_2 \) ratio was assumed to be 6.5 (6.5 mmol ATP·mmol \( O_2 \)⁻¹).

(d) The ATP resynthesised from oxygen stored in the working muscle’s myoglobin and local capillary blood (1.5-2 mmol \( O_2 \)·kg dry muscle⁻¹) was assumed to be 10 mmol ATP·kg dry muscle⁻¹ (Harris et al., 1975; Blei et al., 1993). This contribution was assumed to take place during the first 10 s of each sprint (Medbo and Tabata, 1989), and to be the same for each sprint.

(e) The \( \dot{V}O_2 \) before each sprint (pre-sprint \( \dot{V}O_2 \)) was subtracted from the \( \dot{V}O_2 \) measured during the sprint, to give the net increase in oxygen consumption. Average pre-sprint \( \dot{V}O_2 \) values were 0.55±0.05 and 0.78±0.05 l·min⁻¹ for sprints 1 and 2, respectively (N.S.; see APPENDIX G).

Although estimation of aerobic energy supply from pulmonary (whole body) \( \dot{V}O_2 \) may not be as accurate as local (working leg) \( \dot{V}O_2 \) determinations, recent studies have shown that changes in whole body \( \dot{V}O_2 \) reflect closely those occurring within the exercising legs both during submaximal and maximal cycling exercise (Poole et al., 1992; Knight et al., 1992).

### 5.2.4. Statistical analysis

One-way or two-way analyses of variance (ANOVA) for repeated measures on both factors were used where appropriate for statistical analysis. Where significant F ratios were found (\( P<0.05 \)), the means were compared using a Tukey post-hoc test. Relationships between variables were examined by calculating the product moment correlation coefficient (\( r \)). Results are presented as mean ± standard error (SE).
5.3. RESULTS

5.3.1. Preliminary tests

The oxygen uptake ($\dot{V}O_2$), respiratory exchange ratio ($R$), heart rate (HR), blood lactate (BLa), percentage $\dot{V}O_2\text{max}$ (%$\dot{V}O_2\text{max}$) and work rate during the 5 stages of the submaximal test and the $\dot{V}O_2\text{max}$ test are shown in Table 5.1. The submaximal exercise intensity (expressed as a percentage of $\dot{V}O_2\text{max}$) corresponding to a blood lactate concentration of 4 mmol·l$^{-1}$ (%4mM) was 68±3 %$\dot{V}O_2\text{max}$. A low and not significant correlation ($r=0.59$) was obtained between %4mM and $\dot{V}O_2\text{max}$ expressed in ml·kg$^{-1}$·min$^{-1}$.

Table 5.1. Oxygen uptake ($\dot{V}O_2$), respiratory exchange ratio ($R$), heart rate (HR), blood lactate (BLa), work rate and percentage $\dot{V}O_2\text{max}$ (%$\dot{V}O_2\text{max}$) during the 5 stages of the submaximal test and the $\dot{V}O_2\text{max}$ test (mean±SE, n=8).

<table>
<thead>
<tr>
<th>Submaximal stage number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>$\dot{V}O_2\text{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>%$\dot{V}O_2\text{max}$</td>
<td>61±2</td>
<td>71±2</td>
<td>80±2</td>
<td>86±2</td>
<td>93±2</td>
<td>100</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (l·min$^{-1}$)</td>
<td>2.70±0.15</td>
<td>3.10±0.14</td>
<td>3.53±0.15</td>
<td>3.78±0.16</td>
<td>3.98±0.12</td>
<td>4.41±0.19</td>
</tr>
<tr>
<td>$R$</td>
<td>0.97±0.05</td>
<td>1.00±0.04</td>
<td>1.01±0.04</td>
<td>1.09±0.02</td>
<td>1.11±0.02</td>
<td>1.15±0.03</td>
</tr>
<tr>
<td>HR (beats·min$^{-1}$)</td>
<td>146±3</td>
<td>163±3</td>
<td>174±3</td>
<td>183±3</td>
<td>190±4</td>
<td>196±4</td>
</tr>
<tr>
<td>BLa (mmol·l$^{-1}$)</td>
<td>3.2±0.3</td>
<td>4.8±0.5</td>
<td>6.8±0.7</td>
<td>9.1±0.9</td>
<td>12.0±1.1</td>
<td>-</td>
</tr>
<tr>
<td>Work rate (W)</td>
<td>178±11</td>
<td>210±12</td>
<td>226±12</td>
<td>239±13</td>
<td>258±13</td>
<td>303±13</td>
</tr>
</tbody>
</table>

5.3.2. Power output

Fig. 5.2 and 5.3 show the power output and pedal speed profiles during sprint 1 and sprint 2. There was no difference between power output or pedal speed between corresponding sprints during the two experimental conditions. For example, peak (PPO) and mean power output (MPO$_{30}$) during sprint 1 were 1380±110 W and 724±34 W in the 30-30 condition and 1389±111 W and 727±36 W in the 30-10 condition. Similarly, power output and pedal speed were identical during sprint 2 for the two conditions (e.g. MPO$_{10}$: 949±55 W and 953±57 W, 30-30 vs 30-10). Therefore, the mean values of the 2 conditions are presented for each sprint.

None of the power output indices had returned to the control (sprint 1) values after the 4 min of passive recovery (P<0.01). The mean power
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output during the second 30 s sprint (MPO30) was 594±17 W, which corresponded to 82±2% of the MPO30 generated during sprint 1. Peak power output during sprint 2 was 1138±69 W (82±2% of sprint 1), while MPO10 and MPO120 were 84±2% and 81±2% of the corresponding sprint 1 values (Fig. 5.2).

As can be seen in Fig. 5.2, about 45% of the total work during the sprint was generated in the first 10 s, and this percentage remained the same for both sprint 1 and sprint 2. The fatigue index was also the same for sprint 1 and sprint 2 (62±3% vs 62±1%, N.S.). The pedal speed parameters and the work during acceleration (Wacc) for both sprints are shown in Table 5.2. Wacc was the parameter which recovered less during sprint 2.

An interesting observation was that the subjects with a higher PPO during sprint 1 had a higher fatigue index during that sprint (r = 0.83; P<0.01) and a lower aerobic fitness, as expressed by the %VO2max corresponding to a blood lactate concentration of 4 mmol·l⁻¹ (r=-0.83; P<0.01). Furthermore, significant negative correlations (r=-0.75 to -0.90, P<0.05 to 0.01) were found between power output during sprint 1 (PPO and MPO10) and the recovery of power and pedal speed during sprint 2 (%PPO, %MPO10, %MPO30, %maxSp, %Sp6, %Sp10). Finally, recovery of the above power and pedal speed indices during sprint 2 was correlated with %4mM (r=0.82 to 0.94, P<0.01) and the recovery of MPO30 and MPO120 was correlated with VO2max expressed in ml·kg⁻¹·min⁻¹ (r=0.81 and r=0.78, P<0.05).
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Fig. 5.3. Pedal speed profiles (average for n=8) for Sprint 1 and Sprint 2, separated by 4 min of passive recovery.

Table 5.2. Maximum pedal speed (maxSp), speed at which peak power was attained (SpPPO), mean pedal speed during the first 10 s (Sp10), last 20 s (SpL20), and 30 s (Sp30), and work during acceleration (Wacc) for sprint 1 and sprint 2 (mean±SE, n=8). Pedal speed in revs·min⁻¹ and Wacc in Joules (J). * P<0.01 from sprint 1.

<table>
<thead>
<tr>
<th></th>
<th>maxSp</th>
<th>SpPPO</th>
<th>Sp10</th>
<th>SpL20</th>
<th>Sp30</th>
<th>Wacc (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPRINT 1</td>
<td>170±7</td>
<td>156±5</td>
<td>154±5</td>
<td>111±3</td>
<td>125±4</td>
<td>942±96</td>
</tr>
<tr>
<td>SPRINT 2*</td>
<td>149±5</td>
<td>138±4</td>
<td>132±3</td>
<td>90±4</td>
<td>104±3</td>
<td>701±55</td>
</tr>
<tr>
<td>% of sprint 1</td>
<td>88±3</td>
<td>89±2</td>
<td>86±2</td>
<td>81±2</td>
<td>83±2</td>
<td>75±5</td>
</tr>
</tbody>
</table>
5.3.3. Muscle metabolites

The muscle metabolite concentrations at rest, immediately after sprint 1, 12±0.6 s before sprint 2, and after the second 10 s and 30 s sprint are shown in Table 5.3. The decrease in muscle glycogen during sprint 1 was ~102 mmol glucosyl units·kg dry muscle⁻¹, compared with only 57 mmol glucosyl units·kg dry muscle⁻¹ during sprint 2. The major part (91%) of the decrease in glycogen during sprint 1 could be accounted for by the accumulation of the measured glycolytic intermediates, pyruvate (Pyr) and lactate (La), while only 75% of the decrease in glycogen could be accounted for during sprint 2. There was no resynthesis of muscle glycogen during the 4 min between sprints. The calculated anaerobic glycogenolytic and glycolytic rates for sprint 1 and sprint 2 are shown in Table 5.4. The rate of anaerobic glycogenolysis and glycolysis during sprint 2 was decreased by 55% and 43%, respectively, compared with sprint 1. This was mainly due to the slow rates during the last 20 s of sprint 2 (Table 5.4). The PCr content of the muscle ~6.6 s after sprint 1 was 17.6±1.0% of the resting value, and PCr was resynthesised to 78.7±3.3% of the resting value (n=7) during the 4 min recovery period (Fig. 5.4). During the first 10 s of sprint 2, PCr dropped rapidly to the post 1 sprint levels with no significant decrease thereafter.

![Graph](image)

Fig. 5.4. Time course of changes in muscle phosphocreatine (PCr), during two sprints separated by 4 min of passive recovery. Sprint 1 was 30 s and sprint 2 was either 10 s or 30 s (sprints are represented by bars). Values are for n=8, except for that before sprint 2 (n=7), and are expressed as a percentage of the resting value. * P<0.01 from resting value; † P<0.01 from post sprint 1; ‡ P<0.01 from pre sprint 2.
The percent resynthesis of PCr (%PCr) was negatively correlated with peak power output during sprint 1 ($\text{MPO}_{10}$, $r=-0.78$ and $\text{MPO}_{30}$, $r=-0.75$), implying that PCr resynthesis was slower in the more powerful subjects. Furthermore, %PCr resynthesis was closely correlated with %4mM (Fig. 5.5), linking aerobic fitness to PCr resynthesis. High correlations were found between the %PCr resynthesis and the mean power output ($\text{MPO}_{10}$) and mean pedal speed ($\text{Sp}_{10}$) during the first 10 s of sprint 2, expressed as a percentage of $\text{MPO}_{10}$ and $\text{Sp}_{10}$ during sprint 1 ($r=0.84$, $P<0.05$ and $r=0.91$, $P<0.01$).

There was a 24% decrease in ATP immediately after sprint 1, but no further changes were seen after recovery or during the second sprint (Table 5.3). Calculated Pi concentration increased to $16.2\pm1.3$ mmol·l muscle water$^{-1}$ after sprint 1, but decreased considerably during the recovery period. During the first 10 s of sprint 2, Pi increased to levels higher than after sprint 1 but no further change was seen at the end of 30 s. Calculated $[\text{H}_2\text{PO}_4^-]$ followed a similar pattern as Pi (Table 5.3). No correlation was found between Pi or $\text{H}_2\text{PO}_4^-$ and power output recovery.

A considerable proportion of Pi released during ATP and PCr breakdown appeared in the form of hexose monophosphates (G1P, G6P and G6P). The large increase of hexose monophosphates, especially G6P, after sprint 1 was attenuated during the second sprint (Table 5.3).

---

**Fig. 5.5. Relationship between the percentage of VO$_{2\text{max}}$ corresponding to a blood lactate concentration of 4 mmol·l$^{-1}$ and the percentage of PCr resynthesis after 4 min of passive recovery following a maximal 30 s sprint (n=7, $P<0.01$).**
Table 5.3. Muscle metabolites in vastus lateralis at rest, immediately after sprint 1, following 4 min of recovery (RECOVERY), and after the second 10 s and 30 s sprint.

<table>
<thead>
<tr>
<th></th>
<th>REST</th>
<th>SPRINT 1</th>
<th>RECOVERY</th>
<th>SPRINT 2 (10 s)</th>
<th>SPRINT 2 (30 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (total)</td>
<td>320.7±14.9</td>
<td>218.4±19.5</td>
<td>240.5±25.5</td>
<td>219.4±23.6</td>
<td>184.0±15.8</td>
</tr>
<tr>
<td>acid insoluble</td>
<td>251.2±12.6</td>
<td>164.8±15.3</td>
<td>192.3±20.6</td>
<td>171.4±20.1</td>
<td>143.6±13.2</td>
</tr>
<tr>
<td>acid soluble</td>
<td>69.5±6.7</td>
<td>53.7±6.9</td>
<td>48.2±7.5</td>
<td>48.0±6.1</td>
<td>40.3±8.8</td>
</tr>
<tr>
<td>PCr</td>
<td>76.5±4.3</td>
<td>13.5±1.4</td>
<td>58.5±2.3</td>
<td>16.2±1.6</td>
<td>9.4±2.4</td>
</tr>
<tr>
<td>Cr</td>
<td>44.2±2.8</td>
<td>107.2±4.1</td>
<td>58.6±3.0</td>
<td>104.3±3.9</td>
<td>111.3±3.7</td>
</tr>
<tr>
<td>Total Creatine</td>
<td>120.7±5.2</td>
<td>120.6±5.2</td>
<td>117.0±4.8</td>
<td>120.5±5.3</td>
<td>120.5±5.6</td>
</tr>
<tr>
<td>Pi†</td>
<td>2.9</td>
<td>16.2±1.3</td>
<td>6.4±1.4</td>
<td>20.1±1.9</td>
<td>20.4±1.7</td>
</tr>
<tr>
<td>H2PO4⁻</td>
<td>1.1±0.02</td>
<td>9.2±0.8</td>
<td>3.4±0.8</td>
<td>12.6±1.3</td>
<td>12.5±1.3</td>
</tr>
<tr>
<td>ATP</td>
<td>27.2±0.8</td>
<td>20.7±1.3</td>
<td>22.2±1.0</td>
<td>20.3±1.3</td>
<td>20.8±1.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.6±0.2</td>
<td>6.5±0.7</td>
<td>7.8±0.5</td>
<td>9.2±1.1</td>
<td>10.5±0.9</td>
</tr>
<tr>
<td>G1P</td>
<td>0.19±0.06</td>
<td>2.8±0.6</td>
<td>0.8±0.1</td>
<td>1.1±0.3</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>G6P</td>
<td>1.4±0.1</td>
<td>26.3±1.7</td>
<td>14.2±0.7</td>
<td>19.4±1.0</td>
<td>22.3±1.3</td>
</tr>
<tr>
<td>F6P</td>
<td>0.22±0.05</td>
<td>8.9±1.5</td>
<td>2.7±0.3</td>
<td>3.8±0.5</td>
<td>5.5±0.7</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.5±0.1</td>
<td>4.1±0.4</td>
<td>1.1±0.2</td>
<td>2.0±0.2</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>5.6±0.9</td>
<td>106.2±4.5</td>
<td>72.8±5.9</td>
<td>106.5±4.6</td>
<td>130.5±4.9</td>
</tr>
</tbody>
</table>

Values are expressed in mmol·kg dry muscle⁻¹ (mean ± SE) for 8 subjects, except column RECOVERY where n=7. Muscle glycogen expressed in mmol glucosyl units·kg dry muscle⁻¹; † Pi and H2PO4⁻, calculated total and diprotonated form of inorganic phosphate (mmol·l muscle water⁻¹). Significant differences: a= P<0.01 from REST; b= P<0.01 from SPRINT 1; c= P<0.01 from RECOVERY; d= P<0.01 from SPRINT 2 (10 s); e= P<0.05 from REST; f= P<0.05 from SPRINT 1; g= P<0.05 from RECOVERY.
5.3.4. Muscle lactate and pH

Muscle lactate increased to 106.2±4.5 mmol·kg dry muscle⁻¹ immediately after sprint 1, and 67±3% (n=7) of that remained in the muscle at the end of the 4 min recovery period. Therefore, subjects started the second sprint with a high muscle lactate content and the accumulation of lactate during that sprint was decreased by 45%, as reflected in the glycolytic rate (Table 5.4). The rate of lactate accumulation during the first 10 s of sprint 2 was 3-fold higher than that during the last 20 s of the same sprint. There was no correlation between muscle lactate at the end of the 4 min recovery period and VO₂max. However, aerobic fitness expressed as the %4mM, was inversely correlated with muscle lactate at the end of the recovery period, expressed as a percentage of the sprint 1 value (r=-0.93, P<0.01; n=7).

Changes in muscle pH before, after, and during the recovery between the two sprints are shown in Fig. 5.6. Muscle pH decreased to 6.71±0.03 immediately after sprint 1 and remained unchanged during recovery (6.80±0.03, N.S.). A further decrease to 6.69±0.03 and 6.61±0.03 was observed after the second 10 s and 30 s sprint. No relationship was found between muscle pH before sprint 2 and %PCr resynthesis or power output recovery. A high correlation was found between muscle pH and La⁺Pyr contents after the sprint 1 (r= -0.83; P<0.01). The slope of that regression equation was 0.006056, which was similar to that found in study (i) (0.005485). Fig. 5.7 shows the relationship between pH and La⁺Pyr for the combined results of the present study and the study in Chapter IV. The in vivo buffering capacity (βvivo) can be estimated from the reciprocal of the slope of that relationship (equivalent to -ΔLa/ΔPyr). The average βvivo for the study in Chapter IV was 182 mmol H⁺·kg dry muscle⁻¹·pH⁻¹, while for the present study it was 165 mmol H⁺·kg dry muscle⁻¹·pH⁻¹. It must be noted that βvivo determined using freeze dried muscle reflects the non-bicarbonate buffering capacity, since most of muscle bicarbonate is lost during freeze drying (Harris et al., 1989; Marlin and Harris, 1991).
Table 5.4. Calculated anaerobic glycogenolytic and glycolytic rates during sprint 1 and sprint 2. Values for sprint 2 are presented as rate for the whole sprint (30 s), rate for the first 10 s and rate for the last 20 s. The ratio of glycogenolysis to glycolysis is also presented for each time interval (values are means±SE, expressed in mmol glucosyl units·kg dry muscle−¹·s−¹; see METHODS for calculations).

<table>
<thead>
<tr>
<th>RATES</th>
<th>Sprint 1</th>
<th>Sprint 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 s</td>
<td>30 s</td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>Glycogenolysis</td>
<td>2.94±0.11</td>
<td>1.34±0.15†</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>1.74±0.07</td>
<td>0.96±0.07‡</td>
</tr>
<tr>
<td>ratio</td>
<td>1.70±0.07</td>
<td>1.38±0.07</td>
</tr>
</tbody>
</table>

* P<0.01 from Sprint 1; † P<0.01 from first 10 s of Sprint 2

Fig. 5.6. Changes in muscle pH during two sprints separated by 4 min of passive recovery. Sprint 1 was 30 s and sprint 2 was either 10 s or 30 s (sprints are represented by bars). Values are for n=8 for rest and post sprint 1, and n=7 for the remaining sampling points. * P<0.01 from resting value; † P<0.05 and ‡ P<0.01 from pre sprint 2 value.
Fig. 5.7. Relationship between muscle pH and muscle lactate + pyruvate (La+Pyr) measured in samples taken immediately after a maximal 30 s sprint. The in vivo buffering capacity was estimated from the reciprocal of the slope of the relationship between pH and La+Pyr, \( \beta_{vivo} = -1 / \text{slope} \). (O) represent samples from the study in Chapter IV and (0) represent samples from the present study.

5.3.5. ATP utilisation

The anaerobic ATP utilisation, as calculated from changes in ATP, PCr, La and Pyr, was 233±9 mmol·kg dry muscle\(^{-1}\) during sprint 1 and was decreased to 139±7 mmol·kg dry muscle\(^{-1}\) during the second sprint, mainly as a result of a 43% decrease in glycolysis (Fig. 5.8). This 40% reduction in the calculated anaerobic ATP utilisation from sprint 1 to sprint 2 was more than twice as high compared with the 18% decrease in mean power output during sprint 2. However, the contribution of aerobic metabolism to energy supply was increased during sprint 2, as indicated by the increase in \( \dot{V}O_2 \) from 2.7±0.1 l·min\(^{-1}\) (sprint 1) to 3.2±0.1 l·min\(^{-1}\) (\( P<0.01 \); see Table 5.5).

The calculated aerobic contribution to ATP resynthesis during sprint 1 was 96±7 mmol·kg dry muscle\(^{-1}\) and was increased to 107±9 mmol·kg dry muscle\(^{-1}\) during sprint 2 (\( P<0.01 \)). Therefore, the sum of calculated ATP utilisation from aerobic and anaerobic metabolism was reduced by 25%, from sprint 1 to sprint 2, which was closer to the 18% decrease in
Table 5.5. Pulmonary ventilation (VE) and oxygen uptake (VO₂), expressed as absolute value and relative to VO₂max) during sprint 1 and sprint 2. Values for sprint 2 are presented as mean for the whole sprint (30 s), mean for the first 10 s and mean for the last 20 s. Values are means±SE (n=8).

<table>
<thead>
<tr>
<th></th>
<th>Sprint 1</th>
<th>Sprint 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 s</td>
<td>first 10 s</td>
</tr>
<tr>
<td></td>
<td>last 20 s</td>
<td></td>
</tr>
<tr>
<td>VE (l·min⁻¹)</td>
<td>110.0±10.0</td>
<td>136.0±7.7 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130.3±16.5 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>138.8±9.3 *</td>
</tr>
<tr>
<td>VO₂ (l·min⁻¹)</td>
<td>2.68±0.10</td>
<td>3.17±0.13 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.96±0.11 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.76±0.12 *</td>
</tr>
<tr>
<td>%VO₂max</td>
<td>61±2</td>
<td>72±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85±3</td>
</tr>
</tbody>
</table>

* P<0.01 from Sprint 1; † P<0.01 from Sprint 2 30 s; ‡ P<0.01 from first 10 s of Sprint.

Fig. 5.8. Calculated ATP utilisation during two 30 s sprints separated by 4 min of passive recovery. Boxes represent the contribution of ATP producing processes to the total ATP utilisation, and values are mmol·kg dry muscle⁻¹. Values are for n=8 for SPRINT 1 and n=7 for SPRINT 2.
mean power. The percentage contribution of aerobic metabolism, anaerobic glycolysis, PCr and ATP breakdown to the total ATP utilisation during sprint 1 and sprint 2 is shown in Table 5.6.

The calculated aerobic and anaerobic ATP utilisation during the first 10 s and the last 20 s of sprint of sprint 2 are shown in Fig. 5.9, and are expressed as a percentage of total ATP utilisation in Table 5.6. The contribution of PCr during the first 10 s of sprint 2 was high, amounting ~43% of the anaerobic or ~34% of the total energy supply. However, PCr concentration was very low at the end of the first 10 s, and its contribution dropped to only 5.5% of total ATP utilisation during the last 20 s of the second sprint. The rate of glycogenolysis during the first 10 s of sprint 2 was positively correlated with the ATP utilisation rate during the same time interval (r=0.88, P<0.01). During the last 20 s of sprint 2, glycolytic rate was reduced to ~1/3 of that calculated for the first 10 s of that sprint. At the same time the percentage aerobic contribution was increased by >3fold to reach 65% of the total ATP utilisation (Table 5.6). High correlations were found between VO\textsubscript{2}max (in ml\cdot kg\textsuperscript{-1} \cdot min\textsuperscript{-1}) and the % aerobic contribution to both sprint 1 (r=0.79, P<0.05) and sprint 2 (r=0.87, P<0.01).

Table 5.6. Percentage contribution of anaerobic glycolysis, PCr and ATP breakdown to the total (aerobic+anaerobic) and anaerobic ATP utilisation during sprint 1 and sprint 2. Values for sprint 2 are presented as mean for the whole sprint (30 s), mean for the first 10 s and mean for the last 20 s. Values are mean±SE.

<table>
<thead>
<tr>
<th></th>
<th>Sprint 1</th>
<th>Sprint 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 s (n=8)</td>
<td>30 s (n=7)</td>
</tr>
<tr>
<td>% of total ATP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>4.0±0.6</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>PCr</td>
<td>19.2±1.0</td>
<td>19.8±1.0</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>47.6±1.0</td>
<td>36.0±1.6</td>
</tr>
<tr>
<td>Aerobic</td>
<td>29.2±1.6</td>
<td>43.1±2.2</td>
</tr>
<tr>
<td>% of anaerobic ATP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>5.7±0.9</td>
<td>2.0±0.6</td>
</tr>
<tr>
<td>PCr</td>
<td>27.1±1.0</td>
<td>34.8±1.8</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>67.2±0.8</td>
<td>63.2±1.5</td>
</tr>
</tbody>
</table>
When the contribution of aerobic metabolism was taken into account, the decrease in the rate of ATP utilisation from the first 10 s to the last 20 s of sprint 2 was ≈50%, which compares favourably to the ≈38% decrease in power output between the same time intervals. The corresponding decrease in anaerobic ATP utilisation was as large as 78% (Fig. 5.9).

5.3.6 Blood lactate and blood pH

Changes in blood lactate and pH during the two experimental conditions are shown in Fig. 5.10. Blood lactate increased to ≈9 mmol·l⁻¹ immediately after sprint 1, and continued to increase during the recovery to reach ≈12 mmol·l⁻¹ before sprint 2. There was no significant difference between the blood lactate concentration immediately after the second 10 s and 30 s sprint. However, blood lactate was significantly lower 3.5 min after the second 10 s sprint compared with the second 30 s sprint (14.3±0.7 vs 16.0±0.8 mmol·l⁻¹, P<0.05).
Fig. 5.10. Blood pH (top) and blood lactate (bottom) at rest and during recovery after either two 30 s sprints (30-30, ○) or a 30 s followed by a 10 s sprint (30-10, ♦). * $P<0.01$ between conditions, 30-30 and 30-10 (interaction, condition vs time, $P<0.01$). Changes over time (main effect, time; $P<0.01$): all values are different from REST ($P<0.01$); † $P<0.01$ from immediately after and 3.5 min after sprint 1 (S1); # $P<0.01$ from immediately after S1. (n=8, mean±SE).
Fig. 5.11. Plasma ammonia at rest and during recovery after either two 30 s sprints (○) or a 30 s followed by a 10 s sprint (●). There was no significant difference in plasma ammonia responses between the two conditions (no main effect, 30-30 vs 30-10; no interaction, condition vs time). Changes over time (main effect, time, P<0.01): all values are different from REST (P<0.01); # P<0.01 from immediately after sprint 1 (S1); † P<0.01 from 3.5 min after S1; ‡ P<0.05 from immediately after sprint 2 (S2). (n=8, mean ±SE).

Blood pH decreased from a resting value of 7.41±0.01 to 7.10±0.03 immediately after sprint 1, and did not change during recovery. Following sprint 2, blood pH showed a further decrease to 7.02±0.03 when the duration of the sprint was 30 s, but did not change when a 10 s sprint was performed (Fig. 5.10). Blood pH and blood lactate were inversely correlated at all sampling points (r=-0.83 to -0.93, P<0.01), but no correlation was found between blood lactate and muscle lactate or blood pH and muscle pH.

5.3.7. Plasma ammonia

There was no significant difference between plasma ammonia responses in the two experimental conditions (Fig. 5.11). Plasma ammonia reached 102±21 μmol·l⁻¹ 3.5 min after sprint 1, and peaked 3.5 min after sprint 2.
5.3.8. Plasma electrolytes, changes in plasma volume and heart rate

The first 30 s sprint resulted in a 15.1±1.4% decrease in plasma volume, which did not recover in the next 3.5 min (Fig. 5.12). Immediately after sprint 2, plasma volume was further decreased by 18.1±1.1% of the resting value with no difference between conditions. An average loss of 14.9±1.2% plasma volume remained 3.5 min after sprint 2 in both conditions.

Changes in plasma sodium and potassium were not different between the two experimental conditions (Fig. 5.13). Correction for changes in plasma
Fig. 5.13. Plasma potassium (top) and sodium (bottom) at rest and during recovery after either two 30 s sprints (○) or a 30 s followed by a 10 s sprint (★). Dashed lines represent values corrected for changes in plasma volume. There was no significant difference in plasma potassium or sodium responses between the two conditions (no main effect, 30·30 vs 30·10; no interaction, condition vs time). Changes over time (main effect, time, P<0.01): all values are different from REST (P<0.01), except plasma potassium concentration, corrected for changes in plasma volume, immediately after sprint 2 (S2); # P<0.01 and § P<0.05 from immediately after sprint 1 (S1); † P<0.01 from 3.5 min after S1; ‡ P<0.01 from immediately after S2.
volume did not significantly affect the pattern of plasma potassium responses, but reversed changes in plasma sodium from an increase to a decrease over time. Plasma potassium showed two peaks immediately after each sprint, both followed by a decrease to levels lower than the resting value (Fig. 5.13). However, the peak after sprint 2 was lower than that after sprint 1. Plasma sodium showed less variation over time, remaining 13-19 mmol·l⁻¹ below the resting value throughout the trials.

Peak heart rate during sprint 1 was 184±4 beats·min⁻¹ in both conditions. During the second 30 s sprint heart rate was 179±4 beats·min⁻¹, which was not statistically different from the sprint 1 value. However, heart rate was significantly lower when the second sprint was 10 s (166±4 beats·min⁻¹).
5.4. DISCUSSION

This study was designed to examine changes in muscle metabolites during sprint 2 and to relate these changes to the recovery of power output. One main finding of the study was that PCR was rapidly broken down reaching the post sprint 1 levels during the first 10 s of sprint 2, with no significant change thereafter (Fig. 5.4). Thus, the contribution of PCR to energy supply during the first 10 s of sprint 2 was large, but was minimised during the remaining 20 s. The rapid PCR degradation at the start of very intense exercise has been observed in several studies (Boobis et al., 1982; Jones et al., 1985; Hultman et al., 1990; Soderlund et al., 1992; Gaitanos et al., 1993), and is consistent with the role of PCR to “buffer” sudden changes in ATP and ADP during times of high ATP turnover (Funk et al., 1989; Wallimann et al., 1992). The high correlations between %PCR resynthesis and recovery of power output indices during the initial 10 s of sprint 2, confirm the suggestions made in the previous study (Chapter IV) which linked PCR resynthesis and power output during the initial seconds of the second sprint performed after 1.5 and 3 min of recovery. At this point it must be noted that the large ATP regeneration rate due to PCR contribution during the first 10 s of a sprint was matched by a high power output, and as a result, ~45% of the total work during the 30 s sprint was generated in these first 10 s.

Further support for the significance of PCR during the initial seconds of repeated 30 s sprints comes from a preliminary report by Trump et al. (1994). By occluding the circulation to one leg during the 4 min recovery between the second and third 30 s sprint, they found that PCR resynthesis in that leg was prevented. As a result, total work in sprint 3 was reduced by 15% in the occluded leg and all that decrease occurred during the first 18 s. No other differences in muscle metabolites existed between the occluded and the leg with the free circulation, apart from the different PCR level before sprint 3.

The important role of PCR is to provide energy at rapid rates and prevent large increases in the products of muscle ATPases (myosin, Ca^{2+} and Na^{+}-K^{+}). Therefore, availability of PCR is not only related to muscle contraction via myosin ATPase, but is also involved in ion fluxes taking place during the excitation-contraction coupling (Wallimann et al., 1992). An
impairment of the Ca$^{2+}$ pump effectiveness due to changes in the free energy of ATP hydrolysis has been identified as one of the causes of slowing of relaxation during fatigue (Westerblad et al., 1991). Recent evidence from isolated sarcoplasmic reticulum vesicles has pointed out the significance of the membrane-bound creatine kinase/PCr system in maintaining concentrations of ATP and ADP in the immediate vicinity of Ca$^{2+}$-ATPase that are favorable to Ca$^{2+}$ pump function (Korge and Campbell, 1994).

From the above, it follows that a high PCr content before a repeated sprint may be important for the ability to generate high power during the initial seconds of the sprint. Therefore, the PCr resynthesis rate of an individual may become a limitation for the ability to recover after sprint exercise. In the present study PCr resynthesis 4 min after sprint 1 was very similar to that predicted from the model in the previous chapter, while muscle pH remained low throughout recovery. In Chapter IV, the individualised modelling approach showed a significant between-subjects variability in the PCr resynthesis rate. It was then hypothesised that this was because of differences in muscle fibre composition and/or endurance training status. In the present study, a high correlation was found between the %PCr resynthesised after 4 min of recovery and the %VO$_{2}$max corresponding to a blood lactate concentration of 4 mmol·l$^{-1}$ (4mM). The 4mM can be taken as a measure of endurance capacity and has been found to be related to the percentage of slow twitch fibres and recovery of peak force after fatigue (Karlsson et al., 1981; Tesch et al., 1985; Foxdal et al., 1994). The correlation between 4mM and %PCr resynthesis would suggest that factors related to endurance capacity also influence PCr resynthesis. Support for this notion comes from studies using phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS), which is a non-invasive method for continuous measurements of phosphate compounds in the muscle. The rate of PCr resynthesis, expressed as the rate constant or the time constant of a monoexponential curve fit on the PCr data, has been taken to reflect the oxidative capacity of the muscle (Chance et al., 1981; Meyer, 1988; Blei et al., 1993; McCully et al., 1991, 1993, 1994). Using this approach, it has been found that the PCr resynthesis rate: (1) is higher in long-distance runners than in controls (Yoshida and Watari, 1993a), (2) improves with endurance training and the adaptations revert in $\approx$40 days with detraining (McCully et al., 1991), (3) is correlated positively with the activity of citrate
synthase (McCully et al., 1993), which in turn parallels capillary density (Tesch et al., 1985) and (4) is reduced in patients with mitochondrial myopathies (Radda et al., 1982; Radda, 1986). A strong link has therefore been established between oxidative capacity and PCr resynthesis, and the data of the present study gives further indirect evidence to support that. An interesting observation was that subjects with the higher power output during sprint 1, had a lower 4mM and a lower PCr resynthesis during recovery.

An additional parameter which is involved in the control of PCr resynthesis after very intense exercise besides aerobic metabolism, is muscle pH (Harris et al., 1976; Sahlin et al., 1979; McCully et al., 1994). However, in this and the previous experiment PCr was significantly resynthesised while muscle pH remained virtually unchanged.

An interesting way to interpret the kinetics of PCr recovery after sprinting may be its dependence on blood flow. It has been shown in several experiments that PCr does not recover when circulation to the limb is occluded after exercise (Harris et al., 1975, 1976; Quistorff et al., 1992; Ren and Hultman, 1989). Furthermore, when blood flow to the recovering muscle is reduced (rather than occluded) either because of disease, as in the case of peripheral vascular disease, or as a result of external pressure (as low as 10 Torr), the rate of PCr resynthesis decreases considerably (Radda, 1986; Blei et al., 1993). This occurs even when muscle pH during the exercise and recovery remains unchanged at resting levels, and suggests that the delivery of oxygen by the local vasculature can slow down the rate of aerobic recovery (Blei et al., 1993). The considerable loss of plasma volume after sprint 1 in the present study (~15%) which persisted throughout recovery is probably related to water movements into the previously exercised muscles (Sjogaard and Saltin, 1982; Kowalchuk et al., 1988a). A combination of a decreased blood pressure (due to blood volume losses) and increased local intramuscular pressure (due to muscle water increase) may reduce blood flow and hence PCr recovery. The increased capillary network and the “less severe” metabolic responses to sprint 1 of the more endurance fit subjects, may result in a better blood flow and greater recovery compared with the more powerful subjects (Tesch and Wright, 1983; Tesch et al., 1985).
While PCr resynthesis is important for power generation during the first 10 s of sprint 2, more than half (53%) of the estimated anaerobic ATP during that period was generated through glycolysis (Fig. 5.9). Regulation of glycogenolysis and glycolysis involves regulation of the flux-generating enzyme, phosphorylase, and the rate-limiting enzyme, phosphofructokinase (PFK), respectively (Newsholme and Leech, 1983). Several studies have shown that the transformation of the inactive \( b \) form of phosphorylase to the active \( a \) form \textit{in vivo} is regulated both at the hormonal level, via adrenaline induced increases in cyclic AMP and at the contractile level by cytoplasmic \( \text{Ca}^{2+} \), inorganic phosphate (Pi), ADP, AMP, and inosine 5'-monophosphate (Chasiotis \textit{et al.}, 1982a, 1983b, Chasiotis, 1983, 1988). On the other hand, PFK is inhibited by high ATP, PCr and citrate, whereas AMP, ADP, Pi, fructose 6-phosphate, fructose 1,6-bisphosphate and ammonia relieve the inhibition (Newsholme and Leech, 1983; Dobson \textit{et al.}, 1986). Both enzyme activities are reduced when pH decreases (Newsholme and Leech, 1983; Chasiotis \textit{et al.}, 1982b), but a significant part of PFK activity is preserved due to increase in the positive modulators of the enzyme (Dobson \textit{et al.}, 1986).

An important finding of the present study was that the glycolytic rate during the first 10 s of sprint 2 was the same as the mean glycolytic rate during sprint 1 (Table 5.4). However, this rate dropped by 3-fold during the last 20 s of sprint 2. The mean glycolytic rate during the whole 30 s period of sprint 2 was \(~55\%\) of that during sprint 1 (Table 5.4). Previous studies have shown that both glycogenolysis and glycolysis were decreased after either \( \approx25\ s \) of electrical stimulation (Spriet \textit{et al.}, 1987b) or repeated 30 s sprints (Spriet \textit{et al.}, 1989) when muscle pH was \( \approx6.7\), which is very similar to the pH measured in the present study before sprint 2. In the study of Spriet \textit{et al.} (1989) glycogenolysis during sprint 2 was similar to that in the present study. However, an attempt to calculate glycolysis during sprint 2 in their study using muscle lactate and pyruvate data gave a very small value (0.58 mmol glucosyl units·kg dry muscle\(^{-1}\)·s\(^{-1}\)). This is probably related to the fact that only 51% of the glycogen broken down during the sprint could be accounted for by glycolytic intermediates, lactate and pyruvate in the muscle. Similarly, calculation of the total anaerobic ATP turnover during that sprint yielded a value which was just over half that.
found in the present study (75 vs 139 mmol ATP·kg dry muscle⁻¹), despite the fact that power output was similar in both studies.

The high glycogenolytic and glycolytic rates during the first 10 s of sprint 2, and their large decline thereafter are not very easy to interpret. Muscle pH remained low during recovery, but it decreased further after 10 and 30 s of sprinting (Fig. 5.6). Therefore an effect of pH on both phosphorylase and PFK is possible. An attractive mechanism for the regulation of both enzymes may be their control by the contraction intensity, reflected in the ATP turnover rate. This notion is based on experiments investigating the control of phosphorylase using electrical stimulation, with simultaneous measurements of phosphorylase activity, glycogenolysis and ATP turnover rate (Ren and Hultman, 1989, 1990). In these studies, it has been shown that muscle can have a high level of active phosphorylase and a high Pi, and yet show no glycogenolytic activity if muscle contraction is absent. A high correlation was observed between glycogenolysis and ATP turnover rate and the authors suggested that factors such as contraction per se and/or increase in free AMP due to the high ATP turnover rate are involved in the regulation of glycogenolysis (Ren and Hultman, 1990). In the present study Pi (both in the monobasic and the dibasic form) were similar after 10 s and after 30 s during sprint 2 (Table 5.3). However, the ATP utilisation rate was at least twice as high during the first 10 s compared with the last 20 s (Fig. 5.9). Furthermore, high correlations were found between the ATP utilisation rate during the first 10 s of sprint 2 and the glycolytic and glycogenolytic rates during that interval. It is therefore possible that factors related to contraction intensity such as an increase in free ADP and AMP and Ca²⁺ may regulate both enzymes. However, ³¹P-MRS studies using occlusion after intense contraction have suggested that free ADP and AMP may be high without any glycogenolysis and glycolysis (Quistorff et al., 1992). Therefore the factor which is intimately linked with contraction intensity and controls the rate of glycogenolysis and glycolysis in vivo is still in question. Part of the answer may be found in single fibre studies. Greenhaff et al. (1994) have shown that during a 30 s sprint on a non-motorised treadmill, glycogenolysis is maximally activated in both fibre types (fast, slow). Since glycogenolytic rate is ~2 times higher in fast compared with the slow twitch fibres (Greenhaff et al., 1993, 1994), a failure to activate the highly glycolytic fast fibres (Lannergren and Westerblad, 1989) will be seen as a decrease of glycogenolysis and glycolysis measured.
in "mixed muscle". This would be due to failure at the level of excitation-contraction and not only as a result of enzymatic control.

If no changes in efficiency have occurred from sprint 1 to sprint 2, the percent decrease of power output should match the total ATP utilisation. When the changes in anaerobic ATP utilisation were taken into account, there was a mismatch between the drop in mean power from sprint 1 to sprint 2 (18%) and the corresponding decrease in anaerobic ATP utilisation (40%). However, oxygen uptake during sprint 2 was increased by ≈19%. A significant part of that discrepancy can be accounted for by the contribution of aerobic metabolism. Calculations of the ATP regenerated by aerobic metabolism were based on oxygen uptake (Hermansen and Medbo, 1984) and the appropriate assumptions (see METHODS). The estimated contribution of aerobic metabolism during sprint 1 in the present study represented almost \( \frac{1}{3} \) (29%) of the total energy supply. This value is in agreement with estimates reported in other studies using either the oxygen deficit method (28%; Withers et al., 1991) or theoretical calculations in combination with power output data (28%; Serresse et al., 1988). The importance of aerobic energy supply was more evident during sprint 2, where oxygen uptake reached 85% \( VO_2\max \) during the last 20 s of the sprint contributing as much as 65% to the total energy supply. Inclusion of the ATP estimates from aerobic metabolism reduced the difference between drop in power and energy supply from sprint 1 to sprint 2, to just 7% (18% drop in power vs 25% drop in total ATP). Considering the assumptions made, this agreement is satisfactory, since a change in muscle efficiency probably occurred from sprint 1 to sprint 2. Muscle efficiency is examined in Chapter VIII, and briefly, involves changes in pedal speed which change efficiency according to the parabolic shape of the power-velocity relationship (Sargeant et al., 1981; Faulkner et al., 1986). The discrepancies between power and ATP utilisation during sprint 2 (from the first 10 s to the last 20 s), can be again explained by both an increase in aerobic metabolism and efficiency, as pedal speed slows down during the last 20 s of the sprint (Table 5.2). In this case the decrease in anaerobic ATP utilisation from the first 10 s to the last 20 s was very large (78%), but was compensated by an equally large increase in aerobic metabolism.
The above findings point to the significant role of aerobic metabolism during repeated 30 s sprints. In a previous study by McCartney et al. (1986) the role of aerobic metabolism was thought to be important during the 3rd and 4th 30 s sprint. However, the present study has provided evidence to show the large contribution by aerobic energy sources during the second 30 s sprint. The high correlations found between VO2max and percent contribution by aerobic metabolism, especially during sprint 2, show the significance of a high aerobic power when performing repeated sprints.

In summary, this study has shown that PCr is very important for high power generation during the first 10 s of a second 30 s sprint performed after 4 min of recovery. Furthermore, aerobic metabolism became increasingly significant during sprint 2, and the percent contribution of aerobic metabolism to energy supply reached 65% during the last 20 s of the sprint. The high relationships found between aerobic fitness and PCr resynthesis and between aerobic power and % aerobic contribution during sprint 2 would suggest that aerobic fitness and power are important for maintaining a high power output during a repeated 30 s sprint.
CHAPTER VI

EFFECTS OF ACTIVE RECOVERY ON POWER OUTPUT 
DURING REPEATED MAXIMAL SPRINT CYCLING

6.1. INTRODUCTION

The energy for a maximal 30 s sprint comes predominantly from anaerobic glycolysis and PCr breakdown (Boobis et al., 1987; Nevill et al., 1989, and Chapter IV) while aerobic metabolism contributes ~28% to the total estimated energy supply (Serresse et al., 1988; Withers et al., 1991, and Chapter V). As suggested in the previous 2 studies, the ability to reproduce power output when sprinting is repeated after a short recovery interval may be related with the incomplete PCr resynthesis and the reduced glycolytic rate possibly due to the low muscle pH (Trivedi and Danforth, 1966; McCartney et al., 1986; Spriet et al., 1987b, 1989).

Following short term high intensity exercise the priorities of the recovering muscle are to restore PCr stores and acid base balance, thereby preparing the energy systems for the next rise in ATP demand. These two processes proceed at different rates as indicated by a half time of ~21-60 s for PCr resynthesis (Harris et al., 1976; Yoshida and Watari, 1993a; McCully et al., 1994; Chapter IV), while muscle lactate and pH recover slower with half times >6-10 min (Sahlin et al., 1976; Allsop et al., 1990; Yoshida and Watari, 1993a; Chapter IV). However, the common regulator for both PCr resynthesis and pH recovery is blood flow, since circulatory occlusion after exercise by means of a cuff prevents PCr resynthesis, pH and lactate recovery (Harris et al., 1975, 1976; Quistorff et al., 1992.). Interestingly, restriction of blood flow during the rest intervals between repeated cycle ergometer sprints also impaired power output recovery (Gaitanos, 1990). Adequate blood flow to the recovering muscle will increase oxygen delivery, and therefore enhance PCr resynthesis (Sahlin et al., 1979), while at the same time lactate (La) and H⁺ will be removed faster due to the greater La and H⁺ gradients between muscle and blood (Sjogaard, 1987; Gladden, 1989). An increased muscle La and H⁺ removal has been shown to result in a faster recovery of muscle performance (Mainwood and
CHAPTER VI: Active recovery and power output during sprinting

Worsley-Brown et al., 1975; Renaud, 1989). Furthermore, removal of H+ from the muscle may enhance PCR resynthesis since \([\text{H}^+]\) affects the creatine kinase equilibrium (Sahlin et al., 1975, 1979).

One way of increasing muscle blood flow during recovery is to perform submaximal exercise with the previously exercised muscles. Many studies examining “active” recovery have focused on the effect of several (>20) minutes of low intensity exercise on blood, as opposed to muscle lactate. The rationale for this approach is that a higher blood flow to the previously exercised muscles will increase lactate output from muscle to the blood, and increase lactate transport to lactate uptake sites such as other muscles or the liver (Gladden, 1989). Additionally, during an “active” recovery La may be oxidised by slow twitch fibres in the previously exercised muscles without appearing in the venous blood (Stanley et al., 1986). Therefore, the result of such a prolonged “active recovery” would be a decrease not only in the muscle lactate (and probably pH) but also a faster decrease in the blood lactate. Indeed, 20-40 min of low intensity “active recovery” (30-60% \(\text{VO}_2\text{max}\)) following exhaustive exercise results in a faster blood lactate disappearance (Hermansen and Stensvold, 1972; Weltman et al., 1979; Weltman and Regan, 1983; Stamford et al., 1981; Dodd et al., 1984). The best exercise intensities for faster blood lactate disappearance were slightly different between studies, but depended on the training status of the subjects, i.e. the best intensity was the highest which could be attained with little or no increase in blood lactate (Hermansen and Stensvold, 1972; Stamford et al., 1978).

Although the effect of an “active” recovery on blood lactate is relatively well documented, only a few studies have examined the impact on subsequent exercise performance. The findings from such studies would seem to suggest that the beneficial effects of an “active” recovery may depend on the intensity of the repeated exercise bout. Thus “active” recovery had no effect on repeated exercise that exhausted the subjects in ~5 min (Weltman et al., 1979; Weltman and Regan, 1983), but improved recovery of maximum sprint cycling lasting 1 min (Weltman et al., 1977) and reduced fatigue during eight 6 s sprints separated by 30 s recovery intervals, compared to passive rest (Signorile et al., 1993). With the exception of the study by Signorile et al., (1993) the duration of the recovery between exercise bouts in all the above experiments was 10 - 20
min (Stamford et al., 1981; Dodd et al., 1984), which would allow full PCr resynthesis and significant muscle pH and lactate recovery (Harris et al., 1976; Sahlin et al., 1976) and therefore weaken the effects of “active” recovery.

It has previously been suggested that “pooling of the blood” in the legs during the rest interval (Spriet et al., 1989) may be related to the slow PCr resynthesis and pH restoration observed after a 30 s sprint (Allsop et al., 1990; Chapter IV). One way to increase local blood flow and assist the recovery processes is to perform low intensity exercise during the recovery period. This may enhance the removal of H⁺ and La from the muscle, and may increase oxygen delivery for the resynthesis of PCr. Both H⁺ removal and PCr resynthesis are important for the recovery of power output during sprint 2, since it was shown (Chapter V) that PCr breakdown and glycolytic energy supply provide the major part of the energy during the second 30 s sprint. The purpose of the present study was to examine the effects of active recovery on power output and blood metabolites when a 30 s sprint was repeated after a short recovery interval. The hypothesis was that an increased blood flow during the active recovery may enhance La/H⁺ efflux and PCr resynthesis and therefore power output restoration during the subsequent 30 s sprint.
CHAPTER VI: Active recovery and power output during sprinting

6.2. METHODS

6.2.1. Subjects

Thirteen male university students volunteered to participate in this study. Their mean (±SD) age, height and body mass were 25±3 years, 179±7 cm and 78±9 kg, respectively. All subjects were recreational athletes.

6.2.2. Experimental procedures and protocol

The modified friction-loaded cycle ergometer (Chapter III) was interfaced with a microcomputer and pedal speed and power output were calculated (Lakomy, 1986). The restraining harness described in Chapter III was used to restrict exercise to the leg muscles during the cycle ergometer sprints. Subjects were familiarised with sprinting by completing three separate sprint practice sessions including short (6-10 s) and 30 s sprints.

6.2.2.1. Preliminary tests

During a preliminary visit, the maximum oxygen uptake (VO₂max) of each subject was determined using a continuous incremental test on the Monark cycle ergometer, as described in Chapter III. On a separate session, subjects performed five continuous 4 min stages of submaximal cycling at work rates corresponding to 40±1%, 51±1%, 62±1%, 74±1% and 84±1% VO₂max. Expired air was collected during the last minute of each stage and duplicate samples of arterialised capillary blood (20 μl each) were taken from a pre-warmed thumb during the last 15 s of each stage for lactate determination. The linear relationship between each individual's VO₂ and work rate during these submaximal intensities (r≥0.999) was used to accurately set the load for the 40% VO₂max active recovery (mean load: 1.9±0.1 kg). The relative intensity (%VO₂max) corresponding to a blood lactate concentration of 4 mmol·l⁻¹ was also determined for each subject by linear interpolation.

6.2.2.2. Main tests

Each subject reported to the laboratory for the two main tests at the same time of the day, which was at least four hours after any meal. The two visits were separated by one week, and testing order was randomly assigned. Subjects were requested to repeat their pre-recorded normal diet
and refrain from any form of intense physical activity for 48 hours prior to each test.

The main tests consisted of two 30 s cycle ergometer sprints separated by 4 min of recovery, followed by a 6 s sprint 11 min after the second 30 s sprint (Fig. 6.1). All sprints were performed against a resistance of 75 g·kg⁻¹ body mass (mean load: 5.9±0.2 kg), from a rolling start of approximately 70 revs·min⁻¹. On one occasion the recovery between the two 30 s sprints was active (A), with the subject pedalling backwards for the first and last 30 s of the 4 min recovery, and forwards at 60 revs·min⁻¹ for the middle 3 min against a resistance corresponding to 40% VO₂max. On the other occasion the recovery between the two 30 s sprints was passive (P), with the subject resting on the cycle ergometer seat throughout the 4 min of recovery (Fig. 6.1). After the end of sprint 2 on both occasions, subjects remained seated on the cycle ergometer until the 6 s sprint was performed 11 min later. To attenuate discomfort in the leg muscles between sprints 2 and 3, subjects were allowed to move their legs by slowly turning the pedals backwards for 10 s every minute starting on the 4th min after sprint 2. Strong verbal encouragement was given during each sprint.

Peak power output (PPO), pedal speed at which peak power was attained (SpPPO), maximum pedal speed (maxSp), mean power output for the first
10 s (MPO\textsubscript{10}), the last 20 s (MPO\textsubscript{L20}) and the whole sprint (MPO\textsubscript{30}), and the percentage decline from peak to end power output (fatigue index, FI) were calculated for each sprint. The mean pedal speed during the above time intervals was also calculated (Sp\textsubscript{10}, Sp\textsubscript{L20}, Sp\textsubscript{30}). The work done to accelerate the flywheel to peak speed (during the first 3-4 s of the sprint; W\textsubscript{acc}) was calculated as a measure of the ability of the subject to accelerate.

### 6.2.3 Arterial blood pressure, heart rate and expired air measurements

Heart rate was recorded every minute throughout both tests using short range telemetry (Sports Tester, Polar Electro Fitness, PE3000). Arterial blood pressure (BP) was measured using an automatic blood pressure reading unit (Critikon Inc., Dynmap, Vital signs monitor 1846). This consisted of a cuff which was placed around the upper arm at the level of the heart and was connected to a pressure transducer unit and pump. Measurements were taken by automatic inflation/deflation of the cuff which took ≈20-30 s. Due to the sensitivity of the pressure transducer to arm muscle movements, subjects were instructed to relax their arm muscles during measurements. Arterial blood pressure was measured simultaneously with the blood sampling on the opposite arm (Fig. 6.1).

Expired air samples were collected in Douglas bags during each 30 s sprint and during the middle 2 min of recovery in both conditions (Fig. 6.1). Expired air samples were analysed as previously described.

### 6.2.4 Blood sampling and analysis

On arrival at the laboratory subjects rested on a chair for 20-30 min, while a cannula was placed in an antecubital vein. A 7 ml resting blood sample was then obtained and the subject was moved to the cycle ergometer to start the standardised warm-up. Further blood samples were taken immediately after and 3.5 min after each sprint and 10 min after the second sprint with the subject sitting on the cycle ergometer (Fig. 6.1). Part of the venous sample (=1.5 ml) was placed in a Ca\textsuperscript{2+} heparinised tube for plasma ammonia determination. The remaining blood (=5 ml) was placed in tubes containing lithium-heparin, and blood pH was measured immediately. Blood lactate was determined fluorimetrically at a later date (see Chapter III). Changes in plasma and blood volume were estimated from changes in haematocrit and haemoglobin (Dill and Costill, 1974).
62.5. Statistical analysis

One-way or two-way analyses of variance (ANOVA) for repeated measures on both factors were used where appropriate for statistical analysis. Where significant F ratios were found (P<0.05), the means were compared using a Tukey post-hoc test. Relationships between variables were examined by calculating the product moment correlation coefficient (r). Results are presented as mean ± standard error (SE).
6.3. RESULTS

6.3.1. Preliminary tests

The oxygen uptake (\(\dot{V}O_2\)), respiratory exchange ratio (R), heart rate (HR), blood lactate (BLa), percentage \(\dot{V}O_2_{\text{max}}\) (%\(\dot{V}O_2_{\text{max}}\)) and work rate during the 5 stages of the submaximal test and at \(\dot{V}O_2_{\text{max}}\) are shown in Table 6.1. Blood lactate concentration when cycling at 40±1% \(\dot{V}O_2_{\text{max}}\) was increased from a resting value of 1.0±0.1 to 2.0±0.2 mmol·l\(^{-1}\). The exercise intensity (expressed as a percentage of \(\dot{V}O_2_{\text{max}}\)) corresponding to a blood lactate concentration of 4 mmol·l\(^{-1}\) (%4mM) was 66±2 %\(\dot{V}O_2_{\text{max}}\). No relationship was found between %4mM and \(\dot{V}O_2_{\text{max}}\) expressed in l·min\(^{-1}\) or in ml·kg\(^{-1}\)·min\(^{-1}\).

Table 6.1. Percentage \(\dot{V}O_2_{\text{max}}\) (%\(\dot{V}O_2_{\text{max}}\)), oxygen uptake (\(\dot{V}O_2\)), respiratory exchange ratio (R), heart rate (HR), blood lactate (BLa) and work rate during the 5 stages of the submaximal test and at \(\dot{V}O_2_{\text{max}}\) (mean±SE, n=13).

<table>
<thead>
<tr>
<th>Submaximal stage number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>(\dot{V}O_2_{\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>%(\dot{V}O_2_{\text{max}})</td>
<td>40±1</td>
<td>51±1</td>
<td>62±1</td>
<td>74±1</td>
<td>84±1</td>
<td>100</td>
</tr>
<tr>
<td>(\dot{V}O_2) (l·min(^{-1}))</td>
<td>1.72±0.05</td>
<td>2.16±0.05</td>
<td>2.64±0.08</td>
<td>3.15±0.08</td>
<td>3.59±0.08</td>
<td>4.28±0.13</td>
</tr>
<tr>
<td>R</td>
<td>0.86±0.02</td>
<td>0.90±0.02</td>
<td>0.93±0.01</td>
<td>0.97±0.01</td>
<td>1.01±0.02</td>
<td>1.10±0.01</td>
</tr>
<tr>
<td>HR (beats·min(^{-1}))</td>
<td>117±2</td>
<td>133±3</td>
<td>151±3</td>
<td>167±3</td>
<td>177±2</td>
<td>193±3</td>
</tr>
<tr>
<td>BLa (mmol·l(^{-1}))</td>
<td>2.0±0.2</td>
<td>2.6±0.3</td>
<td>3.4±0.3</td>
<td>5.3±0.3</td>
<td>8.7±0.4</td>
<td>-</td>
</tr>
<tr>
<td>Work rate (W)</td>
<td>114±4</td>
<td>148±5</td>
<td>183±5</td>
<td>218±6</td>
<td>253±7</td>
<td>307±11</td>
</tr>
</tbody>
</table>

6.3.2. Power output

The power output and pedal speed profiles during sprint 1 and sprint 2 for the active (A) and passive (P) recovery conditions are presented in Fig. 6.2 and 6.3. The power output and pedal speed during sprint 1 was the same in the two experimental conditions (e.g. MPO\(_{30}\): 707±25 W and 708±25 W for A and P recovery). Therefore, the performance indices for sprint 1 are presented as the mean values of the two conditions.
Fig. 6.2. Power output profiles for sprint 1 (top) and sprint 2 after 4 min of active or passive recovery (bottom). Boxes represent mean power output (MPO) for the first 10 s and the last 20 s of each sprint. Values are mean±SE for n=13. * P<0.05 from ACTIVE recovery.
Active recovery resulted in significantly higher mean power output during the second sprint, compared with passive recovery (MPO30: 603±17 W and 589±15 W, P<0.05 A vs P recovery). This improvement was totally attributed to a 3.1±1.0% higher power generation during the initial 10 s of the second sprint following the A recovery (P<0.05), since power output during the last 20 s of sprint 2 was the same after both recoveries (Fig. 6.2). A similar improvement was observed in pedal speed parameters after A recovery, with a higher speed during the initial 10 s of sprint 2 (Table 6.2). On average, the improvement in sprint performance recovery after A was between 3 and 4% higher compared with P. All (n=13) except one subject improved their MPO30 during sprint 2 after A. However, four of the subjects had lower PPO recovery in the A compared with the P condition. This was the reason why the 3.5±1.7% improvement in PPO recovery after A was not statistically significant. The average improvement in PPO without these subjects was 6.2±1.4% (P<0.01). A similar effect was observed in Wacc which was the parameter that recovered less during P recovery (79.6±4.4%) but improved by ~8% when the recovery was active (Table 6.2). Again, due to the opposite responses of the 4 subjects, this difference was not statistically significant.

Despite the improved power recovery after active recovery, 4 min of rest were not enough for complete restoration of power output and pedal speed. PPO during sprint 2 was 90±3% and 87±3% of the sprint 1 value after A and P recovery, while the corresponding MPO30 values were ~84 and 86%. The fatigue index was unaffected by both the recovery type (A or P) and sprint number and was 64±2% and 66±2% during sprint 1 and sprint 2. High negative correlations (-0.67 to -0.82; P<0.01) were found in both conditions between power output during the first sprint (PPO, MPO10, MPO30) and the percentage restoration of power and pedal speed during the 30 s and the last 20 s of the second sprint (%MPO30, %Sp30, %MPOL20, %SpL20). However, significant negative correlations (r=-0.56 to -0.60; P<0.05) between power output during sprint 1 and power output and pedal speed restoration during the initial 10 s of sprint 2 (%MPO10 and %Sp10) were found only during the passive recovery condition. This would suggest that although subjects with higher power output during sprint 1 recovered less during sprint 2, the experimental intervention (active recovery) altered this relationship.
Fig. 6.3. Pedal speed profiles (average for n=13) for Sprint 1, and Sprint 2 (S2) after 4 min of active or passive recovery.

Table 6.2. Maximum pedal speed (maxSp), speed at which peak power was attained (SpPPO), mean pedal speed during the first 10 s (Sp10), last 20 s (SpL20), and 30 s (Sp30), and work during acceleration (Wacc) for sprint 1 and sprint 2 during the active (A) and passive (P) recovery conditions. Pedal speed in revs·min⁻¹ and Wacc in Joules (J). All values for sprint 2 are P<0.01 from sprint 1. ** P<0.01 and * P<0.05 from passive recovery (values are mean±SE, n=13).

<table>
<thead>
<tr>
<th></th>
<th>maxSp</th>
<th>SpPPO</th>
<th>Sp10</th>
<th>SpL20</th>
<th>Sp30</th>
<th>Wacc (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPRINT 1 A</td>
<td>169±4</td>
<td>155±4</td>
<td>151±3</td>
<td>110±2</td>
<td>124±2</td>
<td>892±65</td>
</tr>
<tr>
<td>P</td>
<td>170±4</td>
<td>156±4</td>
<td>151±3</td>
<td>110±2</td>
<td>124±2</td>
<td>927±63</td>
</tr>
<tr>
<td>SPRINT 2 A</td>
<td>160±5**</td>
<td>145±5</td>
<td>139±3**</td>
<td>90±2</td>
<td>106±1*</td>
<td>778±64</td>
</tr>
<tr>
<td>P</td>
<td>155±4</td>
<td>143±4</td>
<td>135±2</td>
<td>88±2</td>
<td>103±2</td>
<td>724±52</td>
</tr>
</tbody>
</table>

% improvement
Sprint 2, A vs P 3.6±1.0** 1.8±1.6 3.0±0.7** 1.7±0.7 2.9±0.6* 7.8±3.0
Another interesting relationship found in this study was between $\dot{V}O_{2\text{max}}$ expressed in ml·kg$^{-1}$·min$^{-1}$ and percentage restoration of power output during sprint 2 in both conditions ($r=0.64$, $P<0.05$ to 0.79, $P<0.01$). Similar relationships were observed between the %$\dot{V}O_{2\text{max}}$ corresponding to a blood lactate concentration of 4 mmol·l$^{-1}$ and power restoration during sprint 2 ($r=0.62$, $P<0.05$ to 0.77, $P<0.01$). These correlations would suggest that aerobic fitness and aerobic power are related to the ability to repeat bouts of sprint exercise.

Twelve out of the 13 subjects performed the 6 s sprint (sprint 3) on the 11th min of recovery following sprint 2 during the active recovery condition. However, only 9 subjects performed that 6 s sprint during the passive recovery condition due to general discomfort and subjective feeling of local fatigue in the quadriceps muscles. Therefore, data for $n=9$ are presented in Table 6.3. No differences were found between sprint 2 and sprint 3 in both conditions, indicating that 11 min after sprint 2 there was no further recovery in performance.

Table 6.3. Maximum pedal speed ($\text{maxSp}$), peak power output (PPO), mean power output ($\text{MPO}_6$) and mean pedal speed ($\text{Sp}_6$) during the first 6 s of each sprint, and work during acceleration ($\text{Wacc}$) for sprint 1 ($S_1$), sprint 2 ($S_2$) and sprint 3 ($S_3$) during the active and passive recovery conditions. Pedal speed in revs·min$^{-1}$, power in Watts and Wacc in Joules (J). ** $P<0.01$ and * $P<0.05$ from passive recovery. Values are means±SE, $n=9$.

<table>
<thead>
<tr>
<th>RECOVERY</th>
<th>maxSp</th>
<th>PPO</th>
<th>$\text{MPO}_6$</th>
<th>$\text{Sp}_6$</th>
<th>$\text{Wacc}$ (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTIVE</td>
<td>$S_1$</td>
<td>$165\pm6$</td>
<td>$1297\pm92$</td>
<td>$970\pm53$</td>
<td>$150\pm5$</td>
</tr>
<tr>
<td></td>
<td>$S_2$</td>
<td>$158\pm7^{**}$</td>
<td>$1195\pm106$</td>
<td>$907\pm50^{*}$</td>
<td>$143\pm5^{*}$</td>
</tr>
<tr>
<td></td>
<td>$S_3$</td>
<td>$154\pm6$</td>
<td>$1124\pm108$</td>
<td>$895\pm49$</td>
<td>$140\pm5$</td>
</tr>
<tr>
<td>PASSIVE</td>
<td>$S_1$</td>
<td>$165\pm5$</td>
<td>$1291\pm88$</td>
<td>$971\pm50$</td>
<td>$151\pm4$</td>
</tr>
<tr>
<td></td>
<td>$S_2$</td>
<td>$153\pm6$</td>
<td>$1157\pm103$</td>
<td>$880\pm42$</td>
<td>$139\pm4$</td>
</tr>
<tr>
<td></td>
<td>$S_3$</td>
<td>$155\pm7$</td>
<td>$1129\pm105$</td>
<td>$895\pm50$</td>
<td>$141\pm5$</td>
</tr>
</tbody>
</table>

All values for sprint 2 and 3 are $P<0.01$ from sprint 1. No significant differences were found between values for sprint 2 and 3.
6.3.3. Blood metabolites

Despite the higher power output during sprint 2 after active recovery, no differences were observed between conditions in blood lactate and blood pH (Fig. 6.4). The changes in blood lactate and pH were greater after sprint 1 than after sprint 2. Blood lactate increased from $1.2\pm0.1\text{ mmol}\cdot\text{l}^{-1}$ at rest to $9.0\pm0.6\text{ mmol}\cdot\text{l}^{-1}$ immediately after sprint 1 ($P<0.01$), and continued to increase during the recovery to reach $12.6\pm0.5\text{ mmol}\cdot\text{l}^{-1}$ before sprint 2 ($P<0.05$). After sprint 2, blood lactate was increased to $=17\text{ mmol}\cdot\text{l}^{-1}$ ($P<0.01$) and remained unchanged until the end of the observation period (i.e. 10 min after sprint 2).

Blood pH changes showed a pattern similar to that of lactate, with the largest decrease occurring immediately after sprint 1 (Fig. 6.4). High negative correlations ($r=-0.78$ to $-0.90$; $P<0.01$) were found between blood lactate and pH for all corresponding sampling points with the exception of the resting samples. No relationships were observed between blood lactate or pH before sprint 2 and power output recovery.

Plasma ammonia concentration after sprint 1 increased similarly during both conditions (Fig. 6.5). However, the pattern was different after sprint 2 with the peak ammonia concentration (3.5 min after sprint 2) being significantly higher in the A condition ($205\pm23\text{ vs }170\pm20\text{ \mu mol}\cdot\text{l}^{-1}$; $P<0.05$; Fig. 6.5). Ammonia concentration was correlated with both the corresponding lactate and pH in both conditions ($r=0.61$ to $0.91$, $P<0.01$, ammonia and lactate; and $r=-0.42$, NS to $-0.81$, $P<0.01$, ammonia and pH).
Fig. 6.4. Blood pH (top) and blood lactate (bottom) at rest and during recovery after two 30 s sprints separated by active (○) or passive (♦) recovery. There was no significant difference in blood lactate or pH responses between the two conditions (no main effect, active vs passive; no interaction, condition vs time). Changes over time (main effect, time; P<0.01): all sampling points are different from REST (P<0.01); *P<0.01 from immediately after sprint 1 (S1); § P<0.05 and † P<0.01 from 3.5 min after S1.
Fig. 6.5. Plasma ammonia at rest and during recovery after two 30 s sprints (S1 and S2) separated by active (○) or passive (●) recovery. There was no significant difference between the two conditions (no main effect, active vs passive). Results of Tukey post-hoc test (interaction, condition vs time; P<0.05): all sampling points are different from REST (P<0.01); * P<0.01 and # P<0.05 from immediately after S1; † P<0.01 from 3.5 min after S1; ‡ P<0.05 from immediately after S2; letter (a): difference between active and passive recovery at that sampling point.

Fig. 6.6. Changes in plasma volume during recovery from sprint 1 (S1) and sprint 2 (S2), in the active (■) and passive (■) recovery conditions. There was no significant difference between the two conditions (no main effect, active vs passive). Results of Tukey post-hoc test (interaction, condition vs time; P<0.05): * P<0.01 and # P<0.05 from immediately after S1; † P<0.01 from 3.5 min after S1; ‡ P<0.01 from immediately after S2; § P<0.01 from 3.5 min after S2.
6.3.4. Changes in plasma volume, arterial blood pressure and heart rate

No differences were found between active and passive recovery in changes in plasma volume (Fig. 6.6). The first 30 s sprint resulted in a 14.0±0.9% decrease in plasma volume, which did not recover in the next 3.5 min (Fig. 6.6). A further decrease in plasma volume to 18.1±1.1% (A) and 19.7±1.2% (P) of the resting value was observed after sprint 2, with no significant difference between conditions. The calculated loss of total blood volume was 8.4±0.7% and 7.3±0.7% immediately after and 3.5 min after sprint 1, and reached the highest (~11%) immediately after sprint 2. No difference was seen between conditions in blood volume losses. Significant losses in blood and plasma volume persisted 10 min after sprint 2.

Fig. 6.7 shows the changes of systolic, diastolic and mean arterial blood pressure. There was no difference in blood pressure responses between the active and passive recovery conditions. The systolic blood pressure increased from 17.4±0.5 kPa (130±4 mm Hg) to 20.7±1.5 kPa (155±11 mm Hg) after sprint 1 and remained elevated during the 4 min of recovery. A drop in systolic blood pressure was seen immediately after sprint 2 which was followed by an increase 3.5 min after sprint 2. Systolic blood pressure returned to resting levels 10 min after sprint 2.

The magnitude of changes in the diastolic blood pressure was less, with a significant decrease from 11.5±0.5 kPa (87±3 mm Hg) before sprint 2 to 9.4±0.4 kPa (71±6 mm Hg) after sprint 2. Although there was a tendency for a lower than resting diastolic blood pressure 10 min after sprint 2, this difference was not statistically significant (Fig. 6.7).

Mean blood pressure, which is the average pressure driving blood into the tissues throughout the cardiac cycle was increased similarly in the A and P conditions from 12.9±0.3 kPa (97±2 mm Hg) at rest to 14.9±0.5 kPa (112±4 mm Hg) 3.5 min after sprint 1. A rapid decrease in mean blood pressure to the resting level was seen immediately after sprint 2 (Fig. 6.7).

Heart rate responses throughout both tests are shown in Fig. 6.8. No differences were found in peak heart rate between conditions or sprints. However, heart rate was significantly higher (by ~18 beats·min⁻¹; P<0.01) in the active recovery condition during the 2nd, 3rd and 4th min separating the two 30 s sprints (Fig. 6.8).
Fig. 6.7. Systolic (SYS) and diastolic (DIA) arterial blood pressure (top) and mean arterial blood pressure (bottom) at rest and during recovery after each 30 s sprint in the active (○ and ☐) or passive (◇ and ♦) recovery condition. There was no significant difference in the response pattern between the two conditions (no main effect, active vs passive; no interaction, condition vs time). Changes over time (main effect, time, P<0.01): * P<0.01 from REST; † P<0.01 and ¥ P<0.05 from immediately after sprint 1 (S1); § P<0.01 and # P<0.05 from 3.5 min after S1; ‡ P<0.01 from 3.5 min after sprint 2 (values are mean±SE for n=6).
Fig. 6.8. Heart rate immediately after the standardised warm-up (WU), 4 min after the warm-up, before and after each sprint and during recovery in the active (C) or passive (•) recovery condition. * P<0.01 between active and passive recovery (interaction, condition vs time). Differences over time (P<0.01) are not shown for clarity (values are mean±SE for n=13).

6.3.5. Oxygen uptake, carbon dioxide output and ventilation during exercise and recovery

Oxygen uptake and carbon dioxide output during sprint 1, sprint 2 and the middle 2 min of recovery for both conditions are shown in Fig. 6.9. The average $\dot{V}O_2$ during the first sprint was 57±1% of $\dot{V}O_2\text{max}$ and was increased during sprint 2 to 64±2% of $\dot{V}O_2\text{max}$ after passive recovery and further to 77±1% of $\dot{V}O_2\text{max}$ after the active recovery (P<0.01). $\dot{V}O_2$ during the recovery interval was lower during the passive (24±1% of $\dot{V}O_2\text{max}$) compared with the active recovery (55±1% of $\dot{V}O_2\text{max}$; P<0.01).

Similar patterns were observed for $\dot{V}CO_2$, with a higher CO$_2$ output during the recovery interval (P<0.01) and sprint 2 (P<0.05) when recovery was active compared with when it was passive. Respiratory exchange ratio (R) values during sprint 1 were 1.31±0.05 (A) and 1.21±0.05 (P) and remained unchanged during sprint 2 (1.04±0.02, A and 1.17±0.08, P). A significantly higher R was observed during the passive rest between
CHAPTER VI: Active recovery and power output during sprinting

Fig. 6.9. Oxygen uptake (left) and carbon dioxide output (right) during sprinting and recovery in the active (■) and passive (□) recovery conditions. ** P<0.01 and * P<0.05 between active and passive recovery; † P<0.01 from sprint 1; ‡ P<0.01 and # P<0.05 from recovery.

springs (1.97±0.03) compared with an unchanged R during active recovery (1.33±0.03, P<0.01). Pulmonary ventilation is shown in Fig. 6.10. Ventilation increased from 115±7 l·min⁻¹ during sprint 1, to 145-150 l·min⁻¹ during sprint 2 with no difference between A and P recovery. Ventilation was lower during recovery in the passive compared with the active recovery condition.
6.4. DISCUSSION

The main finding of this study was that recovery of power output was enhanced when low intensity exercise was performed between sprints. This improved recovery was not associated with a lower blood lactate concentration or higher blood pH before sprint 2, and no correlations were found between lactate and pH before sprint 2 and power recovery. In previous studies decreases in blood lactate during active, in comparison with passive recovery have been observed, but only when the ‘active’ recovery was prolonged (i.e. >20 min; Dodd et al., 1984; Stamford et al., 1981), which would allow more time for lactate “uptake” by other tissues and muscles (Gladden, 1989). The short duration of recovery interval in the present study in combination with the high muscle lactate and H⁺ production (Allsop et al., 1990, Chapters IV and V) would preclude a lowering of blood lactate and H⁺ as a result of active recovery. The fact that blood lactate concentration and pH were the same during both the active and passive recovery may indicate that the rate of “appearance” of lactate and H⁺ in the blood was increased in parallel with the rate of “disappearance” of lactate and H⁺ from the blood, resulting in similar blood lactate and H⁺ concentrations in both conditions (Stainsby and Brooks, 1990; Brooks, 1991). A further explanation for the lack of difference in blood lactate and pH between conditions may be that lactate was oxidised by slow twitch fibres in the quadriceps muscle without appearing in the venous blood (Stanley et al., 1986; Pagliassotti and Donovan, 1990). In support of the increased uptake/utilisation of blood lactate when recovery was active, are the findings by Gladden (1991) that lactate uptake and oxidation by active muscles increases with activity level and is affected by the concentration of lactate in the blood and blood flow (Stainsby and Brooks, 1990; Gladden, 1991).

The site of blood sampling in the present study (arm vein) also introduced a further problem of interpretation of blood data. This is illustrated by the finding that after a 30 s sprint on an isokinetic cycle ergometer, the femoral arteriovenous difference of lactate indicated lactate output while at the same time the forearm arteriovenous difference showed lactate uptake by the inactive arm muscles (Kowalchuk et al., 1988a; Kowalchuk et al., 1988b; Lindinger et al., 1990). Therefore venous samples from the arm reflect the balance between lactate and H⁺ uptake and efflux.
Although blood metabolite data can only offer a picture of the net changes occurring during recovery, it is the metabolic changes in the recovering muscle that will ultimately affect subsequent performance. In the present study active recovery resulted in higher power output and pedal velocity during the first 10 s of sprint 2, compared with passive recovery. Three possible mechanisms may explain this improvement after the active recovery: (i) a higher PCr resynthesis, (ii) a lower muscle lactate and more importantly [H+] and (iii) an increased contribution of aerobic metabolism to energy supply. However, all three mechanisms are related to blood flow.

Muscle blood flow is a parameter which is difficult to measure, especially during active recovery. However, Saltin et al., (1992) using catheterisation of the femoral artery and vein in combination with the thermodilution technique, have observed an increase in the quadriceps muscle blood flow during active recovery (~30% of peak VO2 for the leg) following high intensity knee extension exercise to exhaustion. As a result of that increase in blood flow, lactate release from the previously active muscles was increased by ~12% compared with passive recovery. A positive effect was also shown in the time to exhaustion after active recovery (20% vs 13% lower than bout 1, passive vs active recovery).

In the present study the higher heart rate during the active recovery together with the similar mean blood pressure during active and passive recovery (Fig. 6.7, 6.8) would suggest that blood flow to the legs was higher during the active recovery. This higher blood flow is explained as follows: stroke volume is dependent on venous blood return, which is in turn affected by total blood volume and "muscle pump action". Since the changes in plasma and blood volume were similar during active and passive recovery (Fig. 6.6), it is logical to assume that due to muscle contractions ("muscle pump action") stroke volume was higher during active recovery. Therefore cardiac output (stroke volume x heart rate) was probably higher during active recovery. If total peripheral resistance was similar in the two conditions, then mean blood pressure (cardiac output x total peripheral resistance) would be expected to be higher when cardiac output was higher. However this was not the case, indicating that total peripheral resistance was higher during passive recovery, possibly arising from resistance of flow in the leg muscles. This would indicate that blood
flow to the leg muscles was less during the passive compared with the active recovery interval. A very interesting way to interpret these results comes from the studies of Sjøgaard (1990). By measuring intramuscular pressure and arterial blood pressure during exercise and recovery they argued that according to the Haagen-Poiseuille equation, blood flow is proportional to the difference between mean arterial blood pressure and intramuscular pressure, and inversely proportional to the local vascular resistance (Sjøgaard, 1987). In order for blood to flow through a vessel, intraluminal pressure must exceed extravascular pressure. An increase of intramuscular pressure over blood pressure can mechanically offset any locally mediated vasodilation and greatly restrict blood flow to that area of the muscle. One factor that may increase intramuscular pressure after sprinting is water shifts from the blood to the exercising muscle. During intense exercise total muscle water in the vastus lateralis can increase by \( \approx 15\% \) mainly due to an increase in the extracellular water (Sjøgaard and Saltin, 1982). After a 30 s sprint on an isokinetic cycle ergometer total muscle water 3.5 min into recovery was 0.23 l·kg dry muscle\(^{-1} \) higher than at rest, and remained at these levels for 9.5 min (Kowalchuk et al., 1988a). The magnitude of water shifts from blood to muscle in the present study is indicated by the large changes in plasma volume. The driving mechanisms of these water shifts are probably changes in osmolarity in the muscles due to production of metabolites (e.g. lactate), and increases in blood pressure (filtration) which were probably much higher during sprinting than those measured here during recovery (Miles et al., 1987; Sale et al., 1993). According to Sjøgaard (1987) an increased muscle water will also increase diffusion distances in the interstitial space, and in combination with the lack of muscle pump will slow down metabolic recovery during passive rest. Therefore it can be suggested that increases in muscle water may increase intramuscular pressure and impair blood flow to some areas in the muscle. This must be seen in combination with the large losses of blood volume during this type of exercise which reached 11\% in the present study. This can be translated to a loss of 0.5 litres of blood (if total blood volume is assumed to be 5 litres), and will affect the recovery haemodynamics (Hildebrandt et al., 1992). The lack of increase in systolic blood pressure throughout the test and the drop of mean blood pressure after sprint 2 would indicate a decrease in the “driving force” of blood against an increased intramuscular pressure due to water shifts. The significance of blood flow for recovery of muscle metabolites has been
demonstrated in those studies where circulation to the muscle was occluded. During occlusion recovery of pH, PCR is completely prevented (Sahlin et al., 1975; Harris et al., 1976).

Some insight into the possible mechanisms for the performance improvement only during the first 10 s of sprint 2 after active recovery may be provided by the study in Chapter V. In that study, muscle metabolites were measured after 10 s and at the end of a 30 s sprint repeated after 4 min of passive recovery. The anaerobic ATP turnover rate during the first 10 s of sprint 2 was more than 4 times higher than that during the remaining 20 s of the sprint. Furthermore, the contribution of PCR during sprint 2 was restricted to the first 10 s of the sprint. The high power output during the first 10 s of a 30 s sprint (~45% of total work during the sprint) requires a high ATP turnover rate from anaerobic metabolism. The higher peak of plasma ammonia after sprint 2 during the active recovery may indicate a higher ATP turnover related to the higher power output during the first 10 s of the second sprint. Since the first 10 s are fuelled mainly by PCR (34%) and glycolysis (42%), the increased blood flow during active recovery may have either enhanced PCR resynthesis, or allowed an initial faster glycolytic rate by removing H+, or both. Earlier studies by Sahlin et al. (1979) have indicated that resynthesis of PCR is related both to O2 availability and the transport of H+ out of the muscle. However, biopsy studies in man (Saltin et al., 1992) and in the Thoroughbred horse (Marlin, 1989) have shown that despite a lowering of muscle lactate during active recovery, muscle pH restoration was unaffected. Nevertheless, the possibility of a lower [H+], especially in the fast twitch fibres (Gladden, 1989), before sprint 2 can not be ruled out since the exercise and recovery conditions were different.

The increased VO2 during sprint 2 may offer another explanation for the enhanced power restoration during active recovery, although its effect would be expected during the last seconds of the sprint. However, as shown in Chapter V, aerobic metabolism provided ~21% of the energy during the first 10 s of the second sprint, and an increase after active recovery can not be excluded.

The very slow power recovery when subjects performed the third sprint after 11 min of rest may be related to blood flow. During these 11 min
heart rate was decreased while blood pressure (especially diastolic) tended to decrease below resting levels and blood volume was still low. Since subjects were sitting on the cycle ergometer during this time (no "muscle pump action"), it is possible that "pooling of the blood" to the leg veins occurred (Spriet et al., 1989). Alternatively, an increased intramuscular pressure due to water shifts (plasma volume loss still =14% ) may have decreased local circulation. It is worth mentioning that the subjective feeling of swollen quadriceps peaked =3-5 min after sprint 2 and remained until sprint 3 was performed.

In summary, this study has shown that low intensity submaximal exercise performed between two maximal 30 s sprints improved power output recovery during the initial 10 s of sprint 2. The beneficial effects of active recovery were possibly mediated by an increased blood flow to the previously exercised muscles. Maintenance of a high blood flow, in face of the significant decline in blood volume, may be an important factor affecting recovery from sprint exercise due to the central role of blood flow in the restoration of the large changes in muscle metabolites.
CHAPTER VII

RECOVERY OF POWER OUTPUT AND MUSCLE METABOLISM AFTER 10 s AND 20 s OF MAXIMAL SPRINT EXERCISE IN MAN

7.1. INTRODUCTION

The results of the previous studies in this thesis have consistently shown that $\approx 44\%$ of the total work done during a maximal 30 s sprint is generated during the initial 10 s. Thereafter, the distribution of work is 31\% and 25\% of the total during the periods 10-20 s and 20-30 s, respectively. Moreover, careful examination of the power and pedal speed profiles reveals that the rate of decline is not uniform throughout the sprint. Instead, the rate of decline in pedal speed and power is initially faster and becomes slower as the sprint progresses.

A possible explanation for the above observations may be provided by comparing the changes in performance with changes in muscle metabolism and more specifically rates of energy supply. The rate of ATP production from anaerobic sources during a maximal 6 s sprint has been reported to be as high as 10-15 mmol·kg dry muscle$^{-1}$·s$^{-1}$ (Boobis et al., 1987; Gaitanos et al., 1993). However, this rate drops to $\approx 5$ mmol·kg dry muscle$^{-1}$·s$^{-1}$ between the 6th and 30th second of the sprint (Boobis et al., 1987). Similar decreases in anaerobic energy supply were also seen in Chapter V between the first 10 s and the last 20 s of a repeated (2nd) sprint.

The large contribution ($\approx 50\%$) of PCr to energy supply during the initial seconds of a sprint (Gaitanos et al., 1993), in combination with the limited PCr content of the muscle may render PCr availability an important limitation for high power generation. In the study by Gaitanos et al. (1993) the mean rate of PCr degradation during a 6 s cycle ergometer sprint was 7.3 mmol PCr·kg dry muscle$^{-1}$·s$^{-1}$, resulting in a decrease of PCr from $\approx 77$ to 33 mmol·kg dry muscle$^{-1}$. Even though the PCr utilisation rate decreases with exercise duration (Hultman et al., 1990) the PCr content of the muscle may go down to very low levels in the first 10-15 s of a maximum sprint. Indeed, in a study by Jones et al. (1985) using only 2 subjects the PCr concentration after a 10 s sprint was as low as that measured after a 30 s
sprint. However, muscle lactate in their study was almost twice as high after the 30 s sprint compared with after the 10 s sprint. Similar differences in muscle lactate between a 10 s and a 30 s sprint have been reported by Jacobs et al. (1983).

The different time course of changes in muscle lactate and PCr make sprinting for different time durations an attractive model for studying the relationship between changes in power output and muscle metabolites in two ways: (i) within a sprint: the rapid rates of PCr breakdown during the first few (6-10) seconds of maximal sprinting (Gaitanos et al. 1993) would suggest that if the sprint is prolonged, muscle would have to rely almost exclusively on anaerobic glycolysis and aerobic metabolism. The implications from this model would be that by varying sprint duration, the level of muscle acidosis can be 'manipulated' while PCr would be low. (ii) during repeated sprints: after varying levels of muscle acidosis have been achieved by differing sprint durations, the recovery of important metabolites, such as PCr, can be examined. By combining muscle metabolite and power output measurements the relationship between metabolites and muscle function can be assessed.

Despite the potential implications of that model, very few studies have focused on examining muscle metabolism during exercise of very short duration. Only one study exists where the same subjects were measured during sprinting of differing durations (Boobis et al. 1982; n=4). Furthermore, there is a scarcity of muscle metabolite data for short duration sprint exercise (Jones et al. 1985; n=2; Jacobs et al. 1982; only muscle lactate data). Finally, no study has examined recovery of power output and muscle metabolites after sprint exercise of differing durations.

Therefore, the purpose of this study was to examine changes in muscle metabolites and power output during, and after a short recovery following sprint exercise of differing durations. By performing a 10 s sprint on one occasion and a 20 s sprint on another occasion, muscle metabolism 10 s into the first sprint will be examined. Furthermore, muscle PCr may drop at similar levels after 10 and 20 s but muscle lactate is expected to be different. Therefore, recovery of PCr and power output can be examined under different muscle acidity.
7.2. METHODS

7.2.1. Subjects

Eight male university students volunteered to participate in this study. Their age, height and weight (mean±SD) were 26±3 years, 179±6 cm and 77±5 kg, respectively. All subjects were recreational athletes.

7.2.2. Experimental procedures and protocol

The modified friction-loaded cycle ergometer described in Chapter III was used to measure power output and flywheel speed (Lakomy, 1986). A restraining harness was passed around the subject’s waist to restrict exercise to the leg muscles during the cycle ergometer sprints. The subjects were familiarised by completing 3 separate sprint practice sessions.

7.2.2.1. Preliminary tests

The maximum oxygen uptake (\(\dot{V}O_{2\text{max}}\)) of each subject was determined during a preliminary visit using a continuous incremental test on the Monark cycle ergometer (Chapter III). On a separate session, subjects performed four continuous 4 min stages of submaximal cycling at work rates corresponding to 46±2%, 58±3%, 68±2% and 76±3% of \(\dot{V}O_{2\text{max}}\). Expired air was collected during the last minute of each stage and duplicate samples of arterialised capillary blood (20 μl each) were taken from a pre-warmed thumb during the last 15 s of each stage for lactate determination. The relative intensity (%\(\dot{V}O_{2\text{max}}\)) corresponding to a blood lactate concentration of 4 mmol·l\(^{-1}\) (%4mM) was determined for each subject by linear interpolation.

7.2.2.2. Main tests

The two randomly assigned main trials were performed one week apart. Two maximal sprints separated by 2 min of passive recovery on the cycle ergometer were performed during each trial. On one occasion the duration of the first sprint was 10 s (10-30 condition) and on the other 20 s (20-30 condition). The second sprint lasted 30 s on both occasions (Fig. 7.1). The resistive load was 75 g·kg\(^{-1}\) body mass (average load: 5.8±0.1 kg) and each sprint started from a rolling start of approximately 70 revs·min\(^{-1}\).
CHAPTER VII: Metabolic recovery after 10 s and 20 s of sprinting

Each subject performed the two conditions at the same time of day, which was at least four hours after any meal. Diet and exercise were controlled for 2 days prior to each test (Chapter III).

The following performance parameters were obtained for each sprint: peak power output (PPO), pedal speed at which peak power was attained (SpPPO), maximum pedal speed (maxSp), mean power output between 0 and 10 s (MPO10), between 10 and 20 s (MPO10-20) and between 0 and 30 s for sprint 2 only (MPO30). The mean pedal speed during these time intervals was also measured (Sp10, Sp10-20, Sp30). The fatigue index for power output was calculated as the percentage decline from peak to end power output for each sprint. The work done to accelerate the flywheel to peak speed (during the first 3-4 s of the sprint; Wacc) was calculated as a measure of the ability of the subject to accelerate.

On arrival at the laboratory subjects rested on a couch (sitting position) for 30 min, during which time a cannula was placed in an antecubital vein and small incisions through the skin and fascia over the vastus lateralis muscle of both legs were made, under local anaesthesia. A resting blood sample was then obtained followed by the resting biopsy. Only one resting biopsy was obtained during one of the two main trials (balanced randomisation). Further blood samples were taken 1.5 min after sprint 1 and immediately after and 5 min after sprint 2 in each condition (Fig. 7.1). Further biopsies were taken immediately after the first sprint and 11.0±2.1 s before the second sprint on both occasions, while the subject was sitting on the cycle ergometer (Fig. 7.1). The time delay between the end of sprint 1 and the time when the muscle sample was frozen in liquid nitrogen was 6.3±0.5 s. The biopsy leg and testing order were randomised in a balanced design so that biopsies during recovery were taken from the same leg in each condition. Muscle samples were kept in liquid nitrogen until they were freeze dried (within 24 hours of sampling).

Oxygen uptake was measured during each sprint using the Douglas bag technique, and heart rate was recorded every 30 s throughout the test using short range telemetry.
CHAPTER VII: Metabolic recovery after 10 s and 20 s of sprinting

Fig. 7.1. Schematic representation of the experimental design. 
↑, muscle biopsy; ↓, venous blood sample; □, 10 s maximal sprint; 
■, 20 s maximal sprint; ◊, 30 s maximal sprint.

7.2.3. Analytical methods

The freeze dried samples were dissected free of connective tissue and blood and homogenised. At a later date, the following muscle metabolites were determined enzymatically as described in Chapter III: Glycogen, PCr, Cr, ATP, free glucose, G1P, G6P, F6P, Pyr, and La. Muscle metabolite contents (except lactate and glucose) were adjusted to the individual mean total creatine content. Muscle metabolite concentrations are expressed as mmol·kg dry muscle⁻¹.

The mean rates of anaerobic ATP utilisation, glycolysis and glycogenolysis were calculated for sprint 1 as previously described. Changes in inorganic phosphate (Pi) were estimated from changes in the measured phosphate containing metabolites.
Venous blood samples were analysed for blood lactate and plasma ammonia, while blood pH was measured immediately after the sample was taken. Changes in plasma volume were calculated from changes in haematocrit and haemoglobin (Dill and Costill, 1974).

The contribution of aerobic metabolism to energy supply during each sprint was estimated from the VO₂ measurements as detailed in Chapter VI (METHODS).

7.2.4. Statistical analysis

One-way or two-way analyses of variance (ANOVA) for repeated measures on both factors were used where appropriate for statistical analysis. Where significant F ratios were found (P<0.05), the means were compared using a Tukey post-hoc test. Relationships between variables were examined by calculating the product moment correlation coefficient (r). Results are presented as mean ± standard error (SE).
CHAPTER VII: Metabolic recovery after 10 s and 20 s of sprinting

7.3. RESULTS

7.3.1. Preliminary tests

Respiratory parameters, blood lactate (BLa), and work rate during the 4 stages of the submaximal test and the $\dot{V}O_{2max}$ test are shown in Table 7.1. The submaximal exercise intensity corresponding to a blood lactate concentration of 4 mmol·L$^{-1}$ (%4mM) was 69$\pm$2 %$\dot{V}O_{2max}$. No correlation was found between %4mM and $\dot{V}O_{2max}$.

Table 7.1. Oxygen uptake expressed in absolute terms ($\dot{V}O_2$) and as a percentage of $\dot{V}O_{2max}$ (%$\dot{V}O_{2max}$), pulmonary ventilation ($\dot{V}E$), respiratory exchange ratio (R), blood lactate (BLa) and work rate during the 4 stages of the submaximal test and the $\dot{V}O_{2max}$ test (mean±SE, n=8).

<table>
<thead>
<tr>
<th>Submaximal stage number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>$\dot{V}O_{2max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>%$\dot{V}O_{2max}$</td>
<td>46±2</td>
<td>58±3</td>
<td>68±2</td>
<td>76±3</td>
<td>100</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (l·min$^{-1}$)</td>
<td>1.88±0.06</td>
<td>2.39±0.12</td>
<td>2.82±0.10</td>
<td>3.14±0.13</td>
<td>4.16±0.18</td>
</tr>
<tr>
<td>$\dot{V}E$ (l·min$^{-1}$)</td>
<td>40.2±1.9</td>
<td>53.7±3.1</td>
<td>67.4±3.6</td>
<td>80.5±5.1</td>
<td>140.6±2.9</td>
</tr>
<tr>
<td>R</td>
<td>0.84±0.03</td>
<td>0.89±0.03</td>
<td>0.93±0.02</td>
<td>0.96±0.02</td>
<td>1.09±0.03</td>
</tr>
<tr>
<td>BLa (mmol·L$^{-1}$)</td>
<td>1.9±0.2</td>
<td>2.6±0.3</td>
<td>4.0±0.6</td>
<td>5.5±0.8</td>
<td>-</td>
</tr>
<tr>
<td>Work rate (W)</td>
<td>122±5</td>
<td>159±8</td>
<td>189±8</td>
<td>213±10</td>
<td>284±13</td>
</tr>
</tbody>
</table>

7.3.2. Power output

Fig. 7.2 and 7.3 show the power output and pedal speed profiles during sprint 1 and sprint 2 in the two experimental conditions. The duration of sprint 1 was 10 s in one condition and 20 s in the other. Consequently, power output and pedal speed were similar during the initial 10 s of sprint 1 in both experimental conditions. For example, peak power (PPO) and mean power output during the first 10 s of sprint 1 (MPO10) were 1262±46 W and 886±26 W in the 10-30 condition and 1271±55 W and 893±29 W in the 20-30 condition (N.S.). The mean power between 10 and 20 s (MPO10-20) in the 20-30 condition was 643±19 W, which was 28% lower than that during the first 10 s of the sprint. As expected from the longer duration of
the 20 s sprint, the fatigue index (% decline from peak to end power output) was higher compared with the 10 s sprint (56±1% vs 41±2%; P<0.01). Following the 2 min recovery after the 10 s sprint the subjects were able to reproduce the peak power output (PPO) achieved during sprint 1 (Fig. 7.4). However, mean power output during the first 10 s of the second sprint (MPO10) did not recover fully (95±1% of sprint 1;Fig. 7.4). Restoration of PPO and MPO10 following the 20 s sprint was incomplete (89±3% and 88±1% of sprint 1, respectively; Fig. 7.4), and values were significantly lower compared with those after the 10 s sprint (P<0.01). The mean power during the second 30 s sprint after the 10 s and the 20 s sprint was 644±19 W and 592±17 W, respectively (P<0.01). However, the fatigue index during the 30 s sprint was the same in the two conditions (63±1%).

The pattern of change from sprint 1 to sprint 2 for pedal speed variables and Wacc was similar to that observed for the changes in power output (Table 7.2). Both Wacc and SpPPO were fully restored during sprint 2 following the 10 s sprint, but did not return to control values after the 20 s sprint. Peak pedal speed (which was reached 3.5±0.2 s into the sprint in all sprints), Sp10, Sp10-20 and Sp30 did not reach the sprint 1 values in any condition (Table 7.2).
CHAPTER VII: Metabolic recovery after 10 s and 20 s of sprinting

PEDAL SPEED (revs·min⁻¹)

Fig. 7.3. Pedal speed profiles (mean±SE, n=8) for Sprint 1 (●) and Sprint 2 (○), during the 10-30 (left) and the 20-30 condition (right).

Table 7.2. Maximum pedal speed (maxSp), speed at which peak power was attained (SpPPO), mean pedal speed during the first 10 s (Sp₁₀), between 10 and 20 s (Sp₁₀⁻₂₀), between 0 and 30 s (Sp₃₀), and work during acceleration (Wacc) for sprint 1 and sprint 2 in both conditions (after 10 s and after 20 s). Values are mean±SE for n=8.

<table>
<thead>
<tr>
<th></th>
<th>maxSp</th>
<th>SpPPO</th>
<th>Sp₁₀</th>
<th>Sp₁₀⁻₂₀</th>
<th>Sp₃₀</th>
<th>Wacc</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPRINT 1</td>
<td>166±5</td>
<td>151±4</td>
<td>149±4</td>
<td>120±4</td>
<td>-</td>
<td>815±69</td>
</tr>
<tr>
<td>SPRINT 2 (after 10s)</td>
<td>161±4*</td>
<td>148±3</td>
<td>142±3*</td>
<td>111±3*</td>
<td>114±3</td>
<td>765±58</td>
</tr>
<tr>
<td>SPRINT 2 (after 20s)</td>
<td>154±4*</td>
<td>142±3*</td>
<td>133±3*</td>
<td>101±2*</td>
<td>105±2</td>
<td>679±48*</td>
</tr>
</tbody>
</table>

Pedal speed in revs·min⁻¹ and Wacc in Joules (J). * and # P<0.01 and P<0.05 from sprint 1; † all values P<0.01 from SPRINT 2 (10-30). Values for sprint 1 are the mean of the two conditions, except Sp₁₀⁻₂₀ which was measured when sprint 1 was 20 s.
No correlations were found between $\text{VO}_{2\text{max}}$ or %4mM and the recovery of speed or power output. However, subjects who had the higher FI during sprint 1, recovered less during sprint 2 ($r=-0.93$, $P<0.01$, between FI and %PPO recovery).

7.3.3. Muscle metabolites

Table 7.3 shows muscle metabolite concentrations at rest, after sprint 1 and before sprint 2 in the two experimental conditions.

During the first 10 s of sprint 1, muscle glycogen decreased by $46\pm6$ mmol glucosyl units·kg dry muscle$^{-1}$. There was a very good agreement between the calculated rate of glycogenolysis (from changes in La, Pyr and hexose monophosphates) and the measured rate of glycogen degradation (Table 7.4). During the second 10 s period of sprint 1, the rate of muscle glycogen degradation was $\approx40\%$ slower than that during the initial 10 s, and calculated glycogenolysis dropped from $4.4\pm0.4$ to $2.4\pm0.4$ mmol glucosyl units·kg dry muscle$^{-1}$·s$^{-1}$ ($P<0.01$). No resynthesis of muscle glycogen was observed during the 2 min recovery period following the 10 s or the 20 s sprint.

A rapid rate of decrease of PCr ($4.5\pm0.5$ mmol·kg dry muscle$^{-1}$·s$^{-1}$) was observed during the initial 10 s of sprint 1, resulting in a $55\pm2\%$ fall in PCr.
concentration. However, only 14.7±2.6 mmol PCr·kg dry muscle⁻¹ were utilised between the 10th and 20th s of the first sprint, and PCr concentration at the end of 20 s was 27±3% of the resting value. Following 1 min 49 s of recovery after the 10 s and the 20 s sprint, PCr was resynthesised to 86±3% and 76±3% of the resting value, respectively (Table 7.3). These two values were not significantly different, but they were both significantly lower than the resting value (P<0.01).

Changes in calculated Pi generally followed those of PCr. However, a large part of Pi was “trapped” in the hexose monophosphates (HMP: G1P+G6P+F6P) and therefore became unavailable for PCr resynthesis. Inorganic phosphate was increased similarly after both the 10 s and 20 s sprints, and remained ~2 times above the estimated resting value before sprint 2. There was no difference in Pi concentration before sprint 2 between the two conditions (Table 7.3). It is noteworthy that ~21 mmol·kg dry muscle⁻¹ of inorganic phosphate appeared as HMP at the end of recovery following the 20 s sprint (Table 7.3).

All the decrease in ATP during sprint 1 (21±2%) occurred during the first 10 s. No further changes in ATP were seen either after 10 more seconds of sprinting or after 2 min of recovery (Table 7.3).

The high glycolytic rate during the first 10 s of sprint 1 (Table 7.4) resulted in a marked increase in muscle lactate (to 51.0±4.6 mmol·kg dry muscle⁻¹). A significant reduction of the glycolytic rate during the second 10 s of sprint 1 (by ~35%) was reflected as a decrease in muscle lactate accumulation (Table 7.3). Recovery of muscle lactate was slow, with a small drop of ~13-16 mmol lactate·kg dry muscle⁻¹ at the end of the recovery period after both sprints. However, this decrease was only statistically significant in the 20-30 condition. As can be seen in Table 7.3, subjects started the second sprint with high muscle lactate in both conditions, but muscle lactate was almost two times higher when the 20 s sprint preceded the 30 s sprint (Table 7.3). No correlations were found between muscle lactate and the recovery of PCr.

7. 3. 4. ATP utilisation

The anaerobic ATP utilisation during the initial 10 s of sprint 1 was 129±12 mmol·kg dry weight⁻¹ but was decreased to 63±10 mmol·kg dry weight⁻¹ between the 10th and 20th s of sprint 1. This was a result of a 300% decrease in the rate of PCr breakdown and a ~35% decrease in the glycolytic rate.
chapter vii: metabolic recovery after 10 s and 20 s of sprinting

Table 7.3. Muscle metabolites in vastus lateralis at rest, immediately after the 10 s and 20 s sprints, and following 2 min of recovery after the 10 s (REC 10 s), and the 20 s sprints (REC 20 s).

<table>
<thead>
<tr>
<th></th>
<th>REST</th>
<th>10 s SPRINT</th>
<th>20 s SPRINT</th>
<th>REC 10 s</th>
<th>REC 20 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>403.8±20.1</td>
<td>357.4±18.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>329.7±21.4&lt;sup&gt;af&lt;/sup&gt;</td>
<td>364.1±25.0&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>328.4±24.5&lt;sup&gt;a,f,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>insoluble</td>
<td>334.3±13.3</td>
<td>289.0±16.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>272.6±17.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>306.3±23.2</td>
<td>255.6±17.5&lt;sup&gt;a,f,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>soluble</td>
<td>69.5±11.5</td>
<td>68.4±7.9</td>
<td>57.1±7.9</td>
<td>57.8±5.6</td>
<td>72.7±12.4</td>
</tr>
<tr>
<td>Creatine</td>
<td>37.0±3.5</td>
<td>81.8±5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.4±5.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>48.2±3.4&lt;sup&gt;eb,c&lt;/sup&gt;</td>
<td>51.4±3.9&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCr</td>
<td>80.7±3.2</td>
<td>36.1±3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.4±2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>69.5±3.3&lt;sup&gt;e,b,c&lt;/sup&gt;</td>
<td>61.4±2.5&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Cr</td>
<td>117.7±5.0</td>
<td>117.9±3.7</td>
<td>117.8±4.3</td>
<td>117.7±3.9</td>
<td>117.8±4.0</td>
</tr>
<tr>
<td>Pi&lt;sup&gt;†&lt;/sup&gt;</td>
<td>2.9</td>
<td>14.8±1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.4±2.0</td>
<td>5.7±1.0&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>6.9±1.5&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pi (dry)</td>
<td>8.7</td>
<td>44.5±5.3</td>
<td>52.3±6.1</td>
<td>17.1±2.9&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>20.6±4.5&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ATP</td>
<td>25.6±0.7</td>
<td>20.2±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.8±1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.8±1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.8±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.6±0.1</td>
<td>3.0±0.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.6±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5±0.5&lt;sup&gt;a,b,c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>G1P</td>
<td>0.13±0.02</td>
<td>0.85±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.09±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35±0.12&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.30±0.14&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>G6P</td>
<td>1.2±0.1</td>
<td>16.8±1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.5±1.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>9.6±1.0&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>16.7±1.0&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>F6P</td>
<td>0.18±0.03</td>
<td>3.6±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5±0.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.0±0.3&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>3.5±0.2&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>HMP</td>
<td>1.5±0.1</td>
<td>21.2±2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.0±1.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>11.9±1.3&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>20.5±1.1&lt;sup&gt;a,c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.4±0.1</td>
<td>2.8±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8±0.3&lt;sup&gt;a,f&lt;/sup&gt;</td>
<td>1.0±0.2&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.4±0.3&lt;sup&gt;e,b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactate</td>
<td>4.5±0.4</td>
<td>51.0±4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.7±4.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>38.2±2.8&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>66.2±4.8&lt;sup&gt;a,f,g,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed in mmol·kg dry muscle<sup>−1</sup>·(mean±SE) for 8 subjects. Muscle glycogen is expressed in mmol glucosyl units·kg dry muscle<sup>−1</sup>; HMP, hexose monophosphates (G1P+G6P+F6P); †Pi, calculated inorganic phosphate (mmol·l<sup>−1</sup> muscle water); Pi (dry), inorganic phosphate in mmol·kg dry muscle<sup>−1</sup>. Significant differences: <sup>a,c</sup>P<0.01 and <sup>P<0.05</sup> from REST; <sup>b,f</sup>P<0.01 and <sup>P<0.05</sup> from 10 s SPRINT; <sup>c,g</sup>P<0.01 and <sup>P<0.05</sup> from 20 s SPRINT; <sup>d</sup>P<0.01 between REC 10 s and REC 20 s.

Table 7.4. Calculated anaerobic glycogenolytic and glycolytic rates, and measured rate of glycogen degradation during the first 10 s (0-10 s), the second 10 s (10-20 s) and the whole 20 s of sprint 1. The ratio of glycogenolysis to glycolysis is also presented for each time interval.

<table>
<thead>
<tr>
<th></th>
<th>0-10 s</th>
<th>10-20 s</th>
<th>0-20 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogenolysis</td>
<td>4.4±0.4</td>
<td>2.4±0.4&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>2.4±0.2</td>
<td>1.6±0.2&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>ratio</td>
<td>1.8±0.1</td>
<td>1.5±0.1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>rate of ↓ of glycogen</td>
<td>4.6±0.6</td>
<td>2.8±0.5&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.7±0.4</td>
</tr>
</tbody>
</table>

Values are means±SE (n=8), expressed in mmol glucosyl units·kg dry muscle<sup>−1</sup>·s<sup>−1</sup>. **P<0.01 and *P<0.05 from 0-10 s.
Despite this 51% reduction in anaerobic ATP utilisation, the mean power output between 10 s and 20 s of sprint 1 was reduced by only 28% (Fig. 7.5). During the same period, oxygen uptake increased from 1.30±0.15 l·min⁻¹ during the first 10 s to 2.40±0.23 l·min⁻¹, thereby increasing the contribution of aerobic metabolism to energy supply (Table 7.5). A further increase in VO₂ was observed during sprint 2 which was similar in both conditions (2.95±0.15 and 3.02±0.16 l·min⁻¹, N.S.). The calculated aerobic contribution to ATP resynthesis during sprint 1 is shown in Table 7.5, while Table 7.6 shows the percentage contribution of energy systems to the total measured energy utilisation. Aerobic metabolism contributed =13% of the total energy during the first 10 s, and its contribution was doubled during the next 10 s period. The subjects with the higher VO₂max had the higher % aerobic contribution to both sprint 1 (20 s; r=0.79, P<0.05) and sprint 2 (r=0.83, P<0.05). Glycolytic ATP represented =50% of the total energy during both 10 s periods, and the contribution of PCr was only =17% between 10 and 20 s of the sprint (Table 7.6).

When the contribution of aerobic metabolism was taken into account, the decrease in the rate of ATP utilisation from the first 10 s to the second 10 s period of sprint 1 was =40%, which is closer to the =28% decrease in power output between the same time intervals.

Table 7.5. Pulmonary ventilation (VE) and oxygen uptake (VO₂, expressed as absolute value and relative to VO₂max) during the first 10 s (0-10 s), the second 10 s (10-20 s) and the whole 20 s of sprint 1. Values for VE and VO₂ during sprint 2 are the mean of the two conditions. The calculated ATP from aerobic metabolism (mmol·kg dry muscle⁻¹) is also presented for sprint 1.

<table>
<thead>
<tr>
<th></th>
<th>Sprint 1</th>
<th>Sprint 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10 s</td>
<td>10-20 s</td>
</tr>
<tr>
<td>VE (l·min⁻¹)</td>
<td>75.8±12.7</td>
<td>117.2±14.1 *</td>
</tr>
<tr>
<td>VO₂ (l·min⁻¹)</td>
<td>1.30±0.15</td>
<td>2.40±0.23 *</td>
</tr>
<tr>
<td>% VO₂max</td>
<td>32±5</td>
<td>57±4</td>
</tr>
<tr>
<td>aerobic ATP (mmol·kg⁻¹)</td>
<td>20±2</td>
<td>25±3</td>
</tr>
</tbody>
</table>

Values are means±SE (n=8). * P<0.01 from 0-10 s; † P<0.01 from 0-20 s Sprint 1.
Fig. 7.5. Calculated anaerobic ATP utilisation (bottom) and mean power output (MPO; top) during the first sprint. Mean values are given for the intervals between 0-10 s and 10-20 s.
Table 7.6. Percentage contribution of anaerobic glycolysis, PCr and ATP breakdown to the total (aerobic+anaerobic) and anaerobic ATP utilisation during the first 10 s (0-10 s), the second 10 s (10-20 s) and the whole 20 s of sprint 1. Values are means±SE.

<table>
<thead>
<tr>
<th></th>
<th>Sprint 1</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10 s</td>
<td>10-20 s</td>
<td>0-20 s</td>
</tr>
<tr>
<td>% of total ATP</td>
<td>ATP</td>
<td>7.3±1.3</td>
<td>0.9±0.8</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>30.0±1.2</td>
<td>16.7±1.7</td>
</tr>
<tr>
<td></td>
<td>Glycolysis</td>
<td>49.3±2.3</td>
<td>54.0±4.1</td>
</tr>
<tr>
<td></td>
<td>Aerobic</td>
<td>13.4±1.6</td>
<td>28.4±5.5</td>
</tr>
<tr>
<td>% of anaerobic ATP</td>
<td>ATP</td>
<td>8.5±1.6</td>
<td>1.3±1.0</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>34.7±1.6</td>
<td>23.3±1.4</td>
</tr>
<tr>
<td></td>
<td>Glycolysis</td>
<td>56.8±1.8</td>
<td>75.4±4.8</td>
</tr>
</tbody>
</table>

7.3.5. Blood lactate and blood pH

Changes in blood lactate and pH during the two experimental conditions are shown in Fig. 7.6. The increase in blood lactate after both sprint 1 and sprint 2 was greater during the 20-30 condition compared with the 10-30 condition. However, peak blood lactate, observed 5 min after sprint 2, was similar in both conditions (13.8±0.8 vs 14.5±0.7 mmol·l⁻¹, 10-30 vs 20-30 condition).

Decreases in blood pH were different between conditions only during recovery after the first sprint (7.20±0.04 vs 7.27±0.02, 10-30 vs 20-30 condition, P<0.01). A further decrease in blood pH was seen after sprint 2, but no changes occurred thereafter. Blood pH and blood lactate were inversely correlated at all sampling points (r=-0.82 to -0.99, P<0.01), but no correlation was found between blood lactate and muscle lactate.

7.3.6. Plasma ammonia

There was no significant difference in plasma ammonia responses between the two experimental conditions (Fig. 7.7). Plasma ammonia showed no increase after sprint 1. However, a high peak concentration was reached 5 min after sprint 2 (200±25 μmol·l⁻¹). Plasma ammonia was closely related to blood lactate (r=0.86 to 0.91, P<0.01), and blood pH (r=-0.83 to -0.90, P<0.01) only in the samples taken after sprint 2.
Fig. 7.6. Blood pH (top) and blood lactate (bottom) at rest and during recovery after either a 10 s sprint (10-30, *) or a 20 s sprint (20-30, □) followed by a 30 s sprint. * P<0.01 between 10-30 and 20-30 (interaction, condition vs time); † P<0.01 from previous sampling point (main effect, time).
Fig. 7.7. Plasma ammonia at rest and during recovery after either a 10 s sprint (10-30, *) or a 20 s sprint (20-30, □) followed by a 30 s sprint. There was no significant difference in the plasma ammonia response pattern between the two conditions (no main effect, 10-30 vs 20-30; no interaction, condition vs time). Changes over time (main effect, time, P<0.01): † P<0.01 from REST; ‡ P<0.01 from before sprint 2; # P<0.05 from after sprint 2.

Fig. 7.8. Changes in plasma volume before, after, and 5 min following the second 30 s sprint (S2) performed after either a 10 s sprint (□) or a 20 s sprint (□). There was no significant difference in the pattern of changes in plasma volume between the two conditions (no main effect, 10-30 vs 20-30; no interaction, condition vs time). Changes over time (main effect, time, P<0.01): † P<0.01 from pre S 2.
CHAPTER VII: Metabolic recovery after 10 s and 20 s of sprinting

7.7. Changes in plasma volume and heart rate

Plasma volume decreased in a similar manner in both conditions. A 11±1% loss in plasma volume was observed after only 10 s of sprinting. An additional 6% decrease was seen at the end of the second sprint which did not recover in the next 5 min (Fig. 7.8).

The heart rate responses during the two conditions are shown in Fig. 7.9. Peak heart rate at the end of sprint 1 was higher (P<0.01) after the 20 s sprint (184±4 beats·min⁻¹) compared with that after the 10 s sprint (174±3 beats·min⁻¹). During the recovery period after sprint 1, heart rate was higher in the 20-30 condition (P<0.01), but peak heart rate after sprint 2 and the following recovery was the same in the two conditions (184±4 beats·min⁻¹).

Fig. 7.9. Heart rate before, after and during recovery from each sprint (S1 and S2) in the two conditions: 10-30 (●) and 20-30 (○). * P<0.01 between corresponding points in the two conditions (interaction, condition vs time).
7.4. DISCUSSION

This study examined two separate aspects of muscle metabolism and power output during sprinting: development of fatigue during the first sprint, and recovery under different metabolic-environments (higher vs lower muscle lactate). One main finding was that the calculated ATP utilisation rate from anaerobic sources decreased by 51% during the second half (10 to 20 s) of the first 20 s sprint. This was mainly due to the 3-fold decrease of PCr breakdown during the second 10 s compared with the initial 10 s of the sprint (15 vs 45 mmol·kg dry muscle⁻¹), and a 35% decrease in glycolysis (Fig. 7.5). This large decrease in the calculated anaerobic energy supply after only 10 s of sprinting, was associated with a less severe (28%) decrease in mean power output during the same time period. Similar findings have been reported by Gaitanos et al. (1993) during ten 6 s cycle ergometer sprints separated by 30 s of recovery. They found a 27% decline in mean power output from sprint 1 to sprint 10, which was accompanied by a 65% decrease in the calculated anaerobic ATP production.

Two possible explanations may be put forward for this mismatch between the decrease in power output and the calculated energy supply from anaerobic sources. Firstly, the contribution of aerobic energy metabolism may constitute a significant part of energy supply even during an all-out sprint of short duration. Secondly, an increase in efficiency expressed as less energy (ATP utilisation) per unit power may have occurred during the second 10 s of the sprint.

That aerobic metabolism may contribute significantly to energy supply during high intensity exercise of short duration has previously been suggested by Medbo and his colleagues (Medbo and Tabata, 1989, 1993). Various estimations of the aerobic contribution to energy supply during 30 s of sprint cycling have all provided a value of $\approx$28-29% of the total energy (Chapter V, Serresse et al., 1988; Withers et al., 1991). Measurement of the oxygen consumption during the first 10 s and 20 s sprint in this study has showed that $\dot{V}O_2$ was almost doubled from the first to the second 10 s of sprint 1 (Table 7.5). Based on appropriate assumptions (see METHODS, Chapter V), it was estimated that aerobic metabolism contributed $\approx$13% and $\approx$28% of the total energy during the periods 0 to 10 s and 10 to 20 s of the first sprint (Table 7.6). It must be noted that the ATP generated from
oxygen contained in myoglobin and local haemoglobin stores of the working muscles was assumed to have been used during the first 10 s of the sprint).

Although the estimated aerobic contribution during the second 10 s of sprint 1 was increased, it can not totally account for the discrepancy between the decline in ATP utilisation and power output. The issue of efficiency during sprint cycling is complex, especially when sprinting on a friction loaded cycle ergometer. Experiments using isokinetic cycle ergometers, where the pedal speed is constant throughout the sprint, have suggested that there is a parabolic relationship between power and pedal speed (Sargeant et al., 1981; McCartney et al., 1983b, 1985). This implies two things: (i) there is an “optimum” pedal speed at which the highest power is generated and (ii) cycling faster or slower than that speed would result in a reduced power output purely as a result of the shape of the power velocity curve. Furthermore, it has been suggested that the percent contribution of slow twitch fibres to power generation will be high when a muscle of “mixed” composition is contracting at slow speeds (Faulkner et al., 1986). The mean pedal speed during the first 10 s of sprint 1 in the present study was 149±4 revs·min⁻¹ and it dropped to 120±4 revs·min⁻¹, during the second 10 s. This latter value is closer to an optimum value of =110 revs·min⁻¹ suggested by Sargeant et al. (1981) and therefore it can be argued that the remaining 12% discrepancy between the decline in total (aerobic+anaerobic) ATP utilisation and power output can be explained by that improvement in “efficiency”.

An interesting finding of this study was that all the decrease in muscle ATP content occurred during the first 10 s of sprint 1. Loss of ATP during intense muscle contractions occurs when the rate of ADP rephosphorylation (energy provision) fails to keep in pace with the rate of ATP utilisation, and serves to prevent large increases in free ADP and AMP and maintain a high ATP/ADP ratio which is important for cell energetics. The activity of the enzyme AMP deaminase, which catalyses AMP conversion to IMP and ammonia, is increased by free ADP, Pi and \( H^+ \), but availability of substrate (AMP) is the primary stimulus for deamination (Tullson and Terjung, 1991). Although there is no information concerning changes in muscle pH during sprint cycling exercise of very short duration, it is estimated that the possible decrease in muscle pH during the first 10 s of sprinting in the present study was small
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(Gaitanos, 1990). Therefore, the loss of ATP at the beginning as opposed to later in the sprint (when pH was probably lower), was probably related to the extremely high ATP utilisation rates observed (~13 mmol ATP·kg dry muscle⁻¹·s⁻¹) during the first 10 s of the sprint. Comparison with muscle metabolite data from 30 s sprints in this thesis reveals that only a small decrease in ATP occurs between the 10th and 30th s of the sprint, despite the decrease in muscle pH to ~6.7 by the end of a 30 s sprint. However, as seen in this study and as also observed by Boobis et al. (1987) after a 6 s and a 30 s sprint, the rate of ATP utilisation drops dramatically after the initial seconds of a sprint. This finding points to the important role of the ATP utilisation rate in AMP deamination in spite of the lack of severe acidosis. Data from subjects with glycolytic enzyme deficiencies (Sahlin et al., 1990; Bertocci et al., 1991) and from iodoacetate poisoned muscles (Sahlin et al., 1981) support the idea that acidosis is not essential for significant ATP losses (Terjung and Tullson, 1992).

The 35% decrease in glycolysis and 45% decrease in glycogenolysis during the second half of sprint 1 are consistent with a possible inhibition of glycogen phosphorylase and phosphofructokinase (PFK) due to an increased muscle acidosis (Chasiotis et al., 1982; Newsholme and Leech, 1983). A decreased rate of muscle lactate accumulation, indicating reduced glycolysis, has also been observed when a 6-10 s sprint was compared with a 30 s sprint (Boobis et al., 1987; Jacobs et al., 1983; Jones et al., 1985). It is noteworthy that this decrease in glycolysis occured in spite of the elevated positive modulators of PFK such as Pi, F6P, hexose bisphosphates and possibly free ADP and AMP, and a decreased ATP and PCr concentration (Dobson et al., 1986). An alternative, very attractive mechanism that may explain the observed reduction of glycogenolysis and glycolysis in mixed muscle, is an impairment of the excitation-contraction coupling at the level of Ca²⁺ release in the fast twitch/highly glycolytic fibres (Nassar-Gentina et al., 1981; Lannergren and Westerblad, 1989; Westerblad et al., 1991). This "selective fatigue" has been suggested in the past in order to explain reductions in performance and metabolism during electrical stimulation (Hultman et al., 1990) and during repeated sprint exercise (Spriet, 1989; Beelen and Sargeant, 1991, 1993). A decreased contribution from fast twitch fibres may also explain the lack of further decrease in ATP observed between 10 and 20 s of sprinting, since it has been shown that fast twitch fibres possess a higher capacity for AMP deamination than the slow
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twitch fibres (Meyer and Terjung, 1979; Katz et al., 1986b; Tullson and Terjung, 1991).

The second aspect examined by the present study was the recovery of muscle metabolism and power output following a short rest (2 min) period after the 10 s and the 20 s sprint. It was hypothesised that PCr would drop to similar levels after 10 and 20 s of sprinting, and that muscle lactate would be higher after the 20 s sprint. Although a large decrease in PCr was observed after both sprints, PCr was \( \approx 15 \text{ mmol·kg dry muscle}^{-1} \) higher after the 10 s compared with after the 20 s sprint. Interestingly, PCr was resynthesised to the same level in both conditions following the 2 min of rest. Comparison of the number of millimoles of PCr resynthesised after the 10 s and after the 20 s sprint (33±4 vs 40±3 mmol·kg dry muscle\(^{-1}\), respectively) also showed no significant difference. Considering that muscle lactate (and probably \([\text{H}^+]\)) was higher after the 20 s sprint, the similar PCr resynthesis would imply that during this short recovery interval PCr resynthesis was not affected by the extent of muscle acidity observed in this study. This is in agreement with the suggestion that the initial PCr resynthesis is mainly limited by \( \text{O}_2 \) availability, and not pH (Sahlin et al., 1979).

A major finding of the present study was that following the 2 min recovery after the 10 s sprint the subjects were able to reproduce the peak power output (PPO) achieved during sprint 1, despite the elevated muscle lactate and calculated inorganic phosphate (Pi). This shows that increased acidity and Pi have no direct effect on the ability of the fibres to generate maximum power. A similar conclusion was reached by Sahlin and Ren (1989) for the recovery of force. They observed full restoration of the maximum isometric voluntary contraction (MVC) 2 min after fatiguing exercise in spite of a high muscle lactate content. The complete recovery of peak power in the present study is an important finding, because it shows that both components of power output, force and velocity of contraction, had recovered. At this point it should be noted that the speed at which peak power occurred (SpPPO) and the work done to accelerate the flywheel (Wacc) were fully restored during sprint 2, showing that all the parameters of peak muscle performance were unaffected by elevated Pi and \([\text{H}^+]\).

In contrast, the restoration of mean power output (MPO\(_{10}\)) following the 2 min of recovery after the 10 s sprint was not complete (95±1% of sprint 1). Restoration of MPO\(_{10}\) would require similar high rates of ATP
regeneration from glycolysis and PCr, as those seen during the first 10 s of sprint 1. The incomplete resynthesis of PCr before sprint 2 and a possible reduction of glycolysis due to elevated H⁺ are expected to reduce the total ATP regeneration during the first 10 s of sprint 2. Thus, it seems that following recovery from the 10 s sprint, muscle could reproduce its performance during the initial stages of the sprint (PPO generated 2 s into sprint) when PCr and glycolysis were still high. As can be seen in Fig. 7.2, the power output profile during sprint 2 was getting further apart from that during sprint 1 as the sprint progressed. A progressive decrease in the ATP regeneration rate may also explain the finding that maximum speed, which was reached 4 s into the sprint, did not recover fully.

In the 20-30 condition, recovery of all power indices was incomplete and also lower than that after the 10 s sprint. Fig. 7.2 and 7.3 show the power output and pedal speed profiles for sprint 1 and sprint 2 in this condition. It is interesting that PCr and calculated inorganic phosphate were similar before sprint 2 in the two conditions (following the 10 s and the 20 s sprint). However, muscle lactate was 38 and 66 mmol·kg dry muscle⁻¹, respectively. Although no muscle biopsies were taken after sprint 2 to allow a direct assessment of the effects on muscle metabolism, the similar PCr before and the same oxygen consumption during sprint 2 in the two conditions, point to a greater reduction in glycolytic ATP regeneration as a result of a higher muscle acidity after the 20 s sprint.

In summary, this study has shown that the rate of ATP utilisation from anaerobic sources was reduced by ~51% during the second half of a 20 s sprint, as a result of low PCr and a 35% decrease in glycolysis. However, mean power output decreased by only 28% due to a 2-fold increase in VO₂ and improved efficiency of muscle contraction. At the end of the recovery following the 10 s sprint, the ability of the subjects to generate maximum power was unaffected, despite the increased muscle lactate and Pi. However, mean power output during the first 10 s of the second sprint could not be reproduced. Power output during the second 30 s sprint was higher after the 10 s, compared with after the 20 s sprint. Since PCr before, and VO₂ during the 30 s sprints were similar, the lower power output may be related to a reduced glycolytic ATP regeneration due to the higher muscle acidosis.
CHAPTER VIII

EFFECTS OF RESISTIVE LOAD ON POWER OUTPUT DURING INTERMITTENT SPRINT EXERCISE

8.1. INTRODUCTION

In all studies described in this thesis subjects exercised on a friction loaded cycle ergometer (Chapter III). Friction-loaded cycle ergometers of a similar design to that described in Chapter III are widely used to measure the power output generated during sprint exercise of short duration (Cumming, 1974; Ayalon et al., 1974; Bar-Or, 1978; Boobis et al., 1987). However, sprinting against a constant load results in a speed of movement that varies within the sprint and between repeated sprints. Thus, according to the parabolic power-velocity relationship, a part of the fatigue during sprinting may be “non-metabolic”, and simply a result of pedalling faster or slower than an “optimum” speed (Lakomy, 1988). Furthermore, measurement and interpretation of the rate of work against external forces, such as friction, presents unique problems. Two main areas of concern can be identified: (a) accurate measurement of external power output and (b) optimisation of the resistive load against which work is done.

Lakomy (1986, 1988) has identified that the conventional method of power output calculation for friction-loaded ergometers [force (i.e. load) x flywheel velocity] is incorrect during sprinting where acceleration is involved. Therefore, a power output term regarding the rate of change of kinetic energy of the flywheel was added to or subtracted from power generated against friction, depending on whether acceleration or deceleration was occurring (Lakomy, 1986).

To reduce problems of interpretation which emerge when acceleration is involved, isokinetic cycle ergometers have been used to measure external power output during all-out exercise (Sargeant et al., 1981; McCartney et al., 1983a; Beelen et al., 1994). By measuring the peak torque applied on the pedal cranks during maximal cycling at different pedal velocities, linear
torque-velocity and parabolic power-velocity relationships have been observed between \(\approx 25\) and \(170\) revs \(\cdot\) min\(^{-1}\) (Sargeant \textit{et al.}, 1981; McCartney \textit{et al.}, 1983b; Beelen and Sargeant, 1991). Similar relationships between resistive force (load), peak power and peak pedal velocity have been reported for sprinting on a friction loaded cycle ergometer (Nadeau \textit{et al.}, 1983; Vandewalle \textit{et al.}, 1985, 1987a).

The fact that a parabolic relationship between external power and pedal velocity is obtained during a multi-joint activity such as cycling, despite (a) joint angular velocities varying throughout the pedal stroke and (b) the leverage of each muscle changing with joint angle, provides the opportunity to identify an "optimum" pedal velocity for power generation during cycling. Thus an optimum pedal velocity of \(\approx 110\) revs \(\cdot\) min\(^{-1}\) has been suggested by Sargeant \textit{et al.} (1981) for isokinetic "sprint" cycling, but this optimum has been found to vary considerably (120-170 revs \(\cdot\) min\(^{-1}\)) possibly depending on muscle fibre composition (McCartney \textit{et al.}, 1983b; Faulkner \textit{et al.}, 1986).

Optimisation methods for peak power output during sprinting on a friction-loaded cycle ergometer, which involve manipulating the resistive load as the independent variable, have shown that the widely used load of 75 g \(\cdot\) kg\(^{-1}\) body mass may be too low for generation of the highest possible peak and mean power during a 30 s sprint. Both direct determinations (Evans and Quinney, 1981; Nadeau \textit{et al.}, 1983; Dotan and Bar-Or, 1983) and estimations using load-pedal velocity relationships (Nakamura \textit{et al.}, 1985; Vandewalle \textit{et al.}, 1985, 1987b; Winter \textit{et al.}, 1991) have suggested optimal loads ranging between 85 and 130 g \(\cdot\) kg\(^{-1}\) body mass. Usually, the load which gave the highest mean power output for 30 s was slightly lower than that giving the highest peak power.

Although these optimisation methods provide some insight into the relationship between speed and power during sprinting, questions still remain regarding the complex interrelations which influence peak power output during a multi-joint activity where acceleration/deceleration cycles occur within and between successive pedal strokes. Failure to include the work done during the initial rapid acceleration phase can result in large (>30%) underestimation of power output (Lakomy, 1988), which will be greater at high pedal speeds and accelerations. Interestingly, Lakomy (1985)
has shown that peak power output, corrected for flywheel acceleration, was little affected by changes in the resistive load between 55 and 115 g·kg⁻¹ body mass. In contrast to the previous findings using uncorrected power output, the highest peak power was generated against the lighter loads, but the differences were small.

The effect of load on power output generation is of great importance when relating muscle metabolism to fatigue and the subsequent recovery of power output. If the mechanical efficiency of muscle contraction is greater at a certain contraction velocity as shown for rat muscle (Lodder et al., 1991), then pedalling faster or slower than an “optimum” speed will reduce power output according to the power-velocity relationship. Furthermore, changes in the shape of the power-velocity relationship of muscles which may occur due to fatigue (Haan et al., 1989) may influence the relationship between muscle metabolism and power output generation.

Therefore, the purpose of the present study was dual: firstly to examine the effects of different resistive loads on power output during single short sprints and secondly, using the two extreme loads, to examine the fatigue and recovery patterns during maximal intermittent sprinting.
Chapter VIII: Resistive load and intermittent sprint exercise

8.2. METHODS

8.2.1. Subjects

Twenty male university students volunteered to participate in two separate experiments. All subjects were involved in recreational training (3-5 times per week).

8.2.2. Experimental procedures and protocol

A modified friction-loaded cycle ergometer (Chapter III) interfaced with a microcomputer was used to record power output and flywheel speed (Lakomy, 1986). A restraining harness was passed around the subject’s waist to restrict exercise to the leg muscles during the cycle ergometer sprints. The subjects were fully familiarised by completing at least 6-7 separate sprint practice sessions on the cycle ergometer over a period of 3 weeks. Subjects were requested to refrain from any form of intense physical activity for 24 h prior to each test. For both experiments subjects reported to the laboratory after at least 4 hours following a light meal.

8.2.2.1. Experiment 1: Effect of resistive load on power output during single 6 s sprints

During the first experiment twelve subjects (age: 24±2 years, height: 178±7 cm, body mass: 75.3±8 kg, mean ±SD) performed 3 pairs of maximal 6 s sprints in a random order, against loads of 50, 75 and 100 g·kg⁻¹ body mass (2 sprints at each load; mean load: 3.8±0.1 kg, 5.7±0.2 kg and 7.6±0.2 kg, respectively) with 6 min rest between sprints. The best sprint at each load, was used for subsequent analysis. Each sprint started from a rolling start of approximately 70 revs·min⁻¹.

8.2.2.2. Experiment 2: Effect of resistive load on power output during repeated 6 s sprints

In the second experiment, eight subjects (age: 25±3 years, height: 180±5 cm, body mass: 78.8±8 kg) performed seven maximal 6 s sprints separated by 30 seconds of passive recovery on two randomly assigned occasions, 24-48 hours apart (Fig. 8.1). On one occasion the resistive load was 50 g·kg⁻¹ body mass (LOW; mean load: 3.9±0.1 kg) and on the other 100 g·kg⁻¹ body mass
During a preliminary visit, the maximum oxygen uptake ($\text{VO}_2\text{max}$) of each subject was determined using a continuous incremental test on the Monark cycle ergometer, as described in Chapter III. On a separate session, subjects performed five continuous 4 min stages of submaximal cycling at work rates corresponding to 41±1%, 52±1%, 63±1%, 74±1% and 86±1% $\text{VO}_2\text{max}$. Expired air was collected during the last minute of each stage and duplicate samples of arterialised capillary blood (20µl each) were taken from a pre-warmed thumb during the last 15 s of each stage for lactate determination. From this test, the relative intensity (%$\text{VO}_2\text{max}$) corresponding to a blood lactate concentration of 4 mmol·l$^{-1}$ was determined for each subject by linear interpolation.

8.2.3. **Power output calculations**

Power output during sprint cycling on a friction loaded ergometer has been calculated in this thesis according to Lakomy (1986). In this calculation, the work done to accelerate the flywheel is added to the work done against friction (load) to give the total "external" power output. In this study, the power output "uncorrected" for changes in kinetic energy of the flywheel was also calculated ($\text{PPO}_{\text{unc}}$ and $\text{MPO}_{\text{unc}}$). This is merely the product of flywheel velocity (m·s$^{-1}$) and force (load x gravitational acceleration).

![Schematic representation of the experimental protocol for experiment 2. Seven maximal 6 s sprints (┸), separated by 30 s of passive recovery, were performed on two occasions against the HIGH and LOW resistive load.](image-url)
Chapter VIII: Resistive load and intermittent sprint exercise

The following performance parameters were obtained for each sprint: Peak power output and mean power output for each 6 s sprint corrected (PPO and MPO$_6$) and uncorrected (PPO$_\text{unc}$ and MPO$_\text{unc}$) for changes in kinetic energy of the flywheel, pedal speed at which peak power was attained (SpPPO), maximum pedal speed (maxSp), mean pedal speed for each 6 s sprint (Sp$_6$) and the percentage decline from peak to end power output (fatigue index, FI). The work done to accelerate the flywheel to peak speed (during the first 3-4 s of the sprint; Wacc) was calculated as a measure of the ability of the subject to accelerate.

8.2.4. Statistical analysis

One-way or two-way analyses of variance (ANOVA) for repeated measures were used where appropriate for statistical analysis. Where significant F ratios were found (P<0.05), the means were compared using a Tukey post-hoc test. Relationships between variables were examined by calculating the product moment correlation coefficient (r). Results are presented as mean ± standard error (SE).
8.3. RESULTS

8.3.1. Experiment 1

No significant differences were found for the corrected peak power output (PPO) when subjects sprinted against 50, 75 and 100 g·kg⁻¹ body mass. In contrast, corrected mean power output (MPO₆) was significantly different between the three loads (Table 8.1).

The uncorrected peak power output and mean power output (PPO_unc and MPO_unc) were significantly lower compared with the corresponding corrected values (P<0.01; Table 8.1). Both parameters increased significantly with each successive resistive load (P<0.01). An example of corrected and uncorrected power output and pedal speed profiles obtained when sprinting against the lightest and heaviest load is shown in Fig. 8.2. Note the similar corrected PPO despite the large difference in pedal speed.

Table 8.1. Corrected (PPO and MPO₆) and uncorrected (PPO_unc and MPO_unc) peak and mean power output during single 6 s sprints against loads of 50, 75 and 100 g·kg⁻¹ body mass.

<table>
<thead>
<tr>
<th>RESISTIVE LOAD (g·kg⁻¹ body mass)</th>
<th>50</th>
<th>75</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO (W)</td>
<td>1247±44</td>
<td>1293±58</td>
<td>1273±64</td>
</tr>
<tr>
<td>PPO_unc (W)*</td>
<td>704±22</td>
<td>916±33†</td>
<td>1034±45‡</td>
</tr>
<tr>
<td>% uncorrected/corrected</td>
<td>57±1%</td>
<td>71±1%</td>
<td>81±1%</td>
</tr>
<tr>
<td>MPO₆ (W)</td>
<td>818±22</td>
<td>949±33†</td>
<td>1014±40‡</td>
</tr>
<tr>
<td>MPO_unc (W)*</td>
<td>633±21</td>
<td>830±29†</td>
<td>943±37‡</td>
</tr>
<tr>
<td>% uncorrected/corrected</td>
<td>77±1%</td>
<td>88±1%</td>
<td>93±1%</td>
</tr>
</tbody>
</table>

† P<0.01 from 50 g·kg⁻¹ body mass; ‡ P<0.01 from 50 and 75 g·kg⁻¹ body mass. * P<0.01 from corrected values for all loads.

The peak pedal speed (maxSp), the speed at which PPO was generated (2nd sec of the sprint), the mean 6 s speed (Sp₆) and the work done to accelerate the flywheel to peak speed (Wacc) were all higher for the lighter load and decreased as load was increased (P<0.01; Table 8.2). In all subjects the resistive load and maximum pedalling speed were inversely related (P<0.01). The correlation coefficients (r) for the individual relationships between load and the corresponding maximum pedalling rate were 0.998
LOAD: 50 g·kg⁻¹ body mass  

LOAD: 100 g·kg⁻¹ body mass

**Fig. 8.2.** Corrected (□) and uncorrected (○) power output and pedal speed profiles (●) during sprinting against 50 g·kg⁻¹ (left) and 100 g·kg⁻¹ body mass (right) for one of the subjects tested. Arrows indicate the time when peak pedal speed is reached.

To 1.0 (Fig. 8.3). From the individual linear regressions of peak pedal speed on resistive load, the y intercept corresponding to the pedal velocity at zero load can be estimated. This value was 240±4 revs·min⁻¹ (range: 218-261). The resistive load corresponding to zero pedal velocity was also estimated by regression analysis of load on peak pedal speed (18.4±1.0 kg). Since power is the product of force and velocity when acceleration is not occurring, a power - velocity curve was calculated as the product of the peak pedal speed (m·s⁻¹) and resistive load (N) for the observed range of speed and load. A second order polynomial was used to describe the parabolic relationship between power and pedal velocity (Fig. 8.4). According to that fit, the apex of the parabolic relationship corresponded to 1038 W, at a pedal speed of 120 revs·min⁻¹ and against a resistive load of 8.8 kg.
Fig. 8.3. Individual relationships between resistive load and peak pedal speed during short sprints against 3 different loads. Correlation coefficients (r) ranged from 0.998 to 1.0.

Table 8.2. Peak pedal speed (maxSp), average pedal speed at which peak power output was generated (SpPPO), mean speed (Sp6) and work done to accelerate the flywheel to peak speed (Wacc) during single 6 s sprints against loads of 50, 75 and 100 g·kg⁻¹ body mass.

<table>
<thead>
<tr>
<th>RESISTIVE LOAD (g·kg⁻¹ body mass)</th>
<th>50</th>
<th>75†</th>
<th>100‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>maxSp (revs.min⁻¹)</td>
<td>189</td>
<td>163</td>
<td>138</td>
</tr>
<tr>
<td>±3</td>
<td>±3</td>
<td>±3</td>
<td>±4</td>
</tr>
<tr>
<td>SpPPO (revs.min⁻¹)</td>
<td>166</td>
<td>149</td>
<td>130</td>
</tr>
<tr>
<td>±3</td>
<td>±3</td>
<td>±3</td>
<td>±4</td>
</tr>
<tr>
<td>Sp6 (revs.min⁻¹)</td>
<td>170</td>
<td>148</td>
<td>126</td>
</tr>
<tr>
<td>±2</td>
<td>±3</td>
<td>±3</td>
<td>±3</td>
</tr>
<tr>
<td>Wacc (J)</td>
<td>1211</td>
<td>852</td>
<td>574</td>
</tr>
<tr>
<td>±40</td>
<td>±43</td>
<td>±38</td>
<td></td>
</tr>
</tbody>
</table>

† all parameters P<0.01 from 50 g·kg⁻¹ body mass; ‡ all parameters P<0.01 from 50 and 75 g·kg⁻¹ body mass
8.3.2. Experiment 2

Both peak and mean corrected power output were higher than the uncorrected values for every sprint ($P<0.01$; Fig. 8.5). The decline of power during successive sprints was also greater for the corrected power values compared to the uncorrected, irrespective of load. The difference between corrected and uncorrected power output values was the incorporation of the changes in the kinetic energy of the flywheel in the corrected method. When the subjects sprinted against the lighter load, the peak pedal speed attained and the acceleration was greater, while the opposite was the case when the heavy loads were used. This was reflected in the Wacc, which was twice as high in the LOW condition (Table 8.3). Wacc was positively related to peak speed for all loads ($r=0.97$ to 0.98, $P<0.01$).
Corrected PPO and decrease of PPO relative to sprint 1 were similar in the HIGH and LOW load conditions, despite the resistive load being twice as high in the HIGH condition (Fig. 8.5). The pattern of decrease in MPO6 during successive sprints was also similar in both conditions, with 75-80% of MPO6 of sprint 1 being generated during the seventh sprint. However, the absolute values of MPO6 were always higher at the heavier load (Fig. 8.5). It is noteworthy that both PPO and MPO6 did not show any further significant decrease over the last 3 sprints.

**Fig. 8.5.** Corrected (left) and uncorrected (right) power output during seven 6 s sprints against loads of 50 (LOW) and 100 g·kg⁻¹ body mass (HIGH). Line graphs show peak power output generated against HIGH (○) and LOW (●) resistive loads. Bar graphs represent mean power output during each 6 s sprint against HIGH (■) and LOW (□) resistive loads. The differences between HIGH and LOW conditions for corrected and uncorrected mean and peak power, were all significant (main effect, LOW vs HIGH; P<0.01) except for corrected PPO (N.S.). There was no interaction (condition vs sprint number) for either corrected peak or mean power output. Differences over time refer to both mean and peak power (main effect, sprint number; P<0.01). On the right panel, the letters close to the top curve refer to both peak and mean power when the load was HIGH. The letters close to the lower curve show differences over time for both peak and mean power, when the load was LOW. Significant differences: a, P<0.01 from Sprint 1; b, P<0.01 from Sprint 2; c, P<0.01 from Sprint 3; d, P<0.01 from Sprint 4; †, P<0.01 from Sprint 3 for MPO6 only; ‡, P<0.01 from Sprint 4 for MPO6 only.
Fig. 8.6. Peak (line graph) and mean pedal speed (bars) during seven 6 s sprints against loads of 50 (LOW) and 100 g·kg\(^{-1}\) body mass (HIGH). Symbols (○) and (●) represent HIGH resistive loads; (●) and (■) represent LOW resistive loads. The differences between HIGH and LOW conditions for both peak and mean pedal speed were significant (main effect, LOW vs HIGH; P<0.01). There was no interaction (condition vs sprint number) for either peak or mean pedal speed. Differences over time refer to both peak and mean pedal speed (main effect, sprint number; P<0.01): a, P<0.01 from Sprint 1; b, P<0.01 from Sprint 2; c, P<0.01 from Sprint 3; d, P<0.01 from Sprint 4.

Table 8.3. Pedal speed at which peak power output was generated (SpPPO), and work done during acceleration of the flywheel to peak speed (Wacc) during the seven sprints against loads of 50 (LOW) and 100 g·kg\(^{-1}\) body mass (HIGH). Pedal speed is in revs·min\(^{-1}\) and Wacc in Joules.

<table>
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<tr>
<th></th>
<th>Sprint 1</th>
<th>Sprint 2</th>
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<tbody>
<tr>
<td>SpPPO HIGH</td>
<td>132±5</td>
<td>127±4</td>
<td>121±3</td>
<td>116±4</td>
<td>112±3</td>
<td>108±2</td>
<td>106±3</td>
</tr>
<tr>
<td>SpPPO LOW†</td>
<td>174±6</td>
<td>173±6</td>
<td>169±5</td>
<td>163±4</td>
<td>158±4</td>
<td>156±4</td>
<td>153±4</td>
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<tr>
<td>Wacc HIGH</td>
<td>515±70</td>
<td>471±44</td>
<td>387±42</td>
<td>356±40</td>
<td>303±24</td>
<td>265±24</td>
<td>232±19</td>
</tr>
<tr>
<td>Wacc LOW†</td>
<td>1196±106</td>
<td>1149±118</td>
<td>1069±86</td>
<td>982±76</td>
<td>914±77</td>
<td>817±60</td>
<td>804±59</td>
</tr>
</tbody>
</table>

† all values P<0.01 from HIGH (main effect, HIGH vs LOW). There was no interaction (condition vs sprint number) for either SpPPO or Wacc; Changes over time (main effect, sprint number): a, P<0.01 from Sprint 1; b, P<0.01 from Sprint 2; c, P<0.01 from Sprint 3; d, P<0.01 from Sprint 4.
The percent decline of power output within each sprint (fatigue index) was twice as high during the LOW condition in relation to the HIGH condition (25±2% vs 50±2%), indicating that power within a sprint could be maintained more when sprinting against a heavy load. The fatigue index remained unchanged between the first and last sprints.

The mean and peak pedal speeds for the 6 s sprints are shown in Fig. 8.6. Although peak pedal speed during sprint 1 was 196±8 revs·min⁻¹ (LOW) vs 138±6 revs·min⁻¹ (HIGH), the decline in speed with each subsequent sprint occurred in parallel in the two conditions (Fig. 8.6). Mean pedal speed followed similar patterns and for the HIGH load ranged from 127±5 to 100±3 rev·min⁻¹ (sprint 1 to sprint 7) whereas for the LOW load ranged from 177±6 to 152±4 rev·min⁻¹ (Fig. 8.6). The speed at which PPO was generated is shown in Table 8.3. All pedal speed parameters remained unchanged during the last 3 sprints.

The $\dot{VO}_{2}\text{max}$ of the subjects was 4.17±0.12 l·min⁻¹ (53±2 ml·kg⁻¹·min⁻¹), while the submaximal exercise intensity corresponding to a blood lactate concentration of 4 mmol·l⁻¹ (expressed as a percentage of $\dot{VO}_{2}\text{max}$) was 65±3 % $\dot{VO}_{2}\text{max}$. No correlation was found between $\dot{VO}_{2}\text{max}$ or %4mM and the recovery of power output during the LOW condition. However, correlations between $\dot{VO}_{2}\text{max}$ and the recovery of power output were significant ($r=0.76$ to $0.82$, $P<0.05$) when subjects sprinted against the HIGH loads.


8.4. DISCUSSION

The aim of the first part of the present study was to manipulate the resistive load and hence pedal velocity, and observe the effect on power output. Unlike isokinetic systems where pedal speed is constant, all-out sprinting on a friction-loaded cycle ergometer against a constant load results in a range of pedal speeds from start to finish of each sprint. By changing the load, a different range of pedal speeds would be expected. Indeed, the mean and peak pedal speeds attained when sprinting against the 3 loads in the present study showed clear differences (Table 8.2). Despite this difference in pedal speed, the corrected PPO generated against loads ranging from 50 to 100 g·kg\(^{-1}\) body mass was unchanged. This finding initially seems to deviate from the parabolic relationship between power and velocity reported for maximal cycling (Sargeant et al., 1981; McCartney et al., 1983b).

One possible explanation for the similar PPO for all loads examined is provided by studies using isokinetic cycle ergometers. McCartney et al. (1983b, 1985) have repeatedly observed a relative plateau of the power velocity curve between pedal speeds of 120 and 160 revs·min\(^{-1}\). The interactions between muscle mechanics (e.g. muscle length and velocity of muscle contraction), and joint biomechanics (e.g. changes in leverage with joint position) for the 3 joints involved (hip, knee, ankle) would prescribe a single pedal speed when all these factors are optimal (Gulch, 1994). This, in turn, may result in a broad range of pedal speeds where peak power production is relatively constant. Considering that the velocity at which peak power was generated in the present study (130 to 166 revs·min\(^{-1}\)) was similar to the range reported by McCartney et al. (1983b), the above explanation may also hold true for the present study.

Another factor that must be considered is the power output generated during the acceleration phase of the sprint. Acceleration of the flywheel during a maximal sprint is rapid and occurs during the first 3-5 seconds until peak speed is achieved (see Fig. 8.2). During the acceleration phase, each pedal stroke is faster than the previous one, while joint angular velocities (e.g. at the knee) change not only between but within pedal strokes (Sargeant, 1987; Beelen et al., 1994). Since power output in the present study is averaged every second, this would mean that peak power
is the average of 4-6 increasingly faster contractions (pedal strokes) performed in that second. Considering this argument together with the parabolic shape of the power-velocity curve (see Fig. 8.3), one would expect peak power to be affected by load only when load becomes very heavy or very light. In this case all the contractions will be performed at either very low or very high speeds (see Fig. 8.4). The range of SpPPO attained in the present study would suggest that such extreme speeds were not reached when peak power was generated.

A parameter that can modify the "average" power-velocity curve (all subjects combined) is the differences in "optimum" pedal speed between individuals. Subjects with a high percentage of fast twitch fibres have been found not only to have a higher peak power output, but to generate this power at higher speeds compared with individuals who have a high percentage of slow twitch fibres (Thorstensson et al., 1976, Tihanyi et al., 1982). Differences in "optimal" pedal speed during sprinting on a friction-loaded cycle ergometer have been reported between endurance and sprint trained athletes (Vandewalle et al., 1987b). In the present study, the wide range of estimated maximum pedal speed (218 to 261 revs·min⁻¹) gave "optimal" pedal speeds between 109 and 130 revs·min⁻¹ (calculated as 0.5 x maximum pedal speed; Nakamura et al., 1985; Vandewalle et al., 1985; Winter, 1991). This would suggest that selecting the load according to body mass does not guarantee that the same relative pedal speed (in relation to the "optimum") will be reached by each subject. An interesting observation was that out of the 12 subjects, six had the highest PPO when sprinting against 75 g·kg⁻¹ body mass, three achieved the highest PPO when sprinting against 50 g·kg⁻¹ and the other three when sprinting against 100 g·kg⁻¹ body mass. Therefore, combining individual "optimum" pedal speeds in order to construct a "mean" curve may result in a power-velocity curve with a flat and broad apex.

The parabolic power-velocity relationship calculated in this study from the linear regressions of peak pedal speed on resistive load, deserves special attention (Fig. 8.4). An important issue to understand before attempting to interpret the findings of the present study based on muscle mechanics, is

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† Pedal stroke calculation: Speed at PPO (revs·min⁻¹) divided by 60 gives the number of pedal revolutions in that second. This number multiplied by 2 (2 pedal strokes in a revolution) gives the number of pedal strokes or contractions in that second.
the nature of the power-velocity relationship in Fig. 8.4. This curve is the result of multiplying resistive loads (force) with the peak pedal speed achieved at each load (power = force x velocity). A special feature of this curve is therefore, that no acceleration is involved at any point, since peak pedal speed is reached (by definition) when acceleration is zero. Thus, no corrections for acceleration are needed for any of the power output points of the curve. However, these power output values should not be confused with peak power output (PPO) values for each load. As can be seen in Fig. 8.2, PPO is attained before peak speed and takes into account work done to accelerate the flywheel. The power output value that is used to construct the power-velocity curve is indicated by the arrow in Fig. 8.2, and can be defined as power at peak speed. Being “free” of the complex interactions occurring during acceleration the power-velocity curve in Fig. 8.4 can be taken as an indication of the ability of the multi-joint “system” to generate power at each specific pedal speed. This argument is enforced by the fact that the apex of the curve was 120 revs·min⁻¹, which is similar to the “optimal” values reported during isokinetic cycling (Sargeant et al., 1981; McCartney et al., 1983b; Beelen and Sargeant, 1991).

Sprinting against the heaviest load (100 g·kg⁻¹ body mass) resulted in a mean (6 s) pedal speed of 126±3 revs·min⁻¹, which was very close to the calculated “optimal” value, and also resulted in the highest mean power output. In contrast, 20% lower mean power was generated when sprinting against 50 g·kg⁻¹ body mass, despite the 35% higher mean and peak pedal speeds (170±2 revs·min⁻¹ and 189±3 revs·min⁻¹). However, PPO was the same in both conditions, making this a very attractive model to use for the study of the effects of power-velocity relationship on the fatigue process. In experiment 2, subjects repeated sprints against a heavy and a light load (100 and 50 g·kg⁻¹ body mass). Thus, a high mean power was generated during each sprint when the load was high, but fatigue (i.e. percent decline of power with every sprint) was similar to the condition when load and power output were much lower (Fig. 8.5). This simply means that when a heavy load was used (and thus pedal speed was kept closer to a theoretical optimum) the subjects appeared to be generating power more ‘efficiently’.

The issue of “efficiency” and optimum movement frequencies for human work output during intense exercise has recently received attention by Sargeant and his colleagues. By measuring total work output and energy
consumption during 10 s of electrical stimulation of rat muscle in situ they found that efficiency was velocity dependent, in a similar way as the power-velocity relationship (Lodder et al., 1991). In an attempt to explain the mechanism which links power output and velocity of contraction in a muscle of mixed composition (fast and slow twitch fibres), they proposed that the relative contribution of fast-contracting/fast-fatiguing fibres may have been higher at faster velocities. Similar suggestions were put forward to explain the velocity-dependent effect of prior exercise on muscle power during subsequent maximal exercise at different pedalling speeds using an isokinetic cycle ergometer (Beelen and Sargeant, 1991, 1993). Selective fatigue of the faster, fatigue sensitive fibres was assumed to occur at their faster velocities (120 revs·min\(^{-1}\)), which were, however, much slower than the pedal rates when the LOW load was used in the present study (peak speed: 196±8 revs·min\(^{-1}\)). Consequently, a part of the reduction in power output observed when cycling at these extreme pedal speeds during the LOW condition may be explained by selective fatigue of the faster fibres in combination with the shape of the power-velocity relationship (See Fig. 8.4). An integration of these two factors has been demonstrated by Faulkner et al. (1986) using human fast and slow twitch fibres in vitro. They concluded that when all fibres in a mixed muscle are contracting, the slow fibres contribute almost as much power as the fast fibres at very low velocities, only slightly to power at moderate velocities, and not at all at high velocities. If this was the case in the present study, then the 50% lower fatigue index during each sprint (25% vs 50%) and the similar decline in power between sprints when the load was HIGH compared with when it was LOW, may be a combination of pedalling closer to an "optimum" pedal speed and using more slow than fast twitch fibres. In contrast the high fatigue index (50%) in only 6 s of sprinting when the load was LOW, may indicate that pedal speed was corresponding to the "steep" part of the power-velocity relationship, and was therefore "uneconomic". A possible "shift" of the power-velocity relationship to the left which may occur due to fatigue (Haan et al., 1989) will exacerbate this problem.

Unfortunately, only one study has examined muscle metabolism when sprinting at different pedal speeds (Jones et al., 1985). Their subjects performed a maximal 30 s sprint on an isokinetic cycle ergometer at pedal speeds of 60 and 140 revs·min\(^{-1}\). The peak power output was higher but the decline in power during the sprint was steeper at the fast speed compared
with the slow speed. However, the total work done during the 30 s period was the same for the two speeds and so were the changes in muscle metabolites. Measurement of muscle metabolites after 10 s of exercise at the two speeds in 2 subjects again showed no difference in the metabolic responses measured in mixed (homogenised) muscle. Greenhaff et al. (1994) have shown extensive involvement of both fibre types when sprinting for 30 s on a non-motorised sprint treadmill. However, glycogenolytic rate was ~60% higher in the fast twitch fibres and this may be related to a higher acidosis in these fibres. Nevertheless, it remains unknown whether or not sprinting at different pedal speeds causes a differing metabolic response in type I (slow twitch) and type II (fast twitch) fibres.

A parameter that may become increasingly important as pedal speed increases is the time allowed to develop force (Sjogaard, 1978; Harridge and White, 1993). During the LOW load, when very fast pedal speeds are reached, muscles have only a few milliseconds to develop force. For example, during sprint 1 in the LOW condition an average peak pedal speed of 196 revs·min\(^{-1}\) was reached (range: 152-220 revs·min\(^{-1}\)). This pedal speed would allow only ~150 ms for force generation, if force is assumed to be generated from the top to the bottom pedal position. In fact, large forces may be applied on the pedals for an even shorter part of the pedal cycle, considering that peak torque is attained when the pedal is at 90° from the vertical (Sargeant et al., 1981). Such short periods may be too low for full force generation (Harridge and White, 1993).

Another interesting way to view the results of the present study is by looking at the number of muscle contractions during sprinting against HIGH and LOW loads. Fig. 8.6 shows the mean and peak pedal speed for each 6 s sprint in each condition. The number of muscle contractions for each leg can be calculated from the mean pedal speed. Since one muscle contraction is done by each leg for each pedal revolution, the number of contractions during the 6 s period is mean pedal speed/10. The greater number of muscle contractions calculated for the test when a LOW load was used may have influenced efficiency. As shown by Bergstrom and Hultman (1988), during electrical stimulation with different durations of the relaxation/contraction cycle, a contraction pattern with more activations and relaxations gave a faster development of fatigue and
increased the energy cost of contractions. They attributed this to the high energy cost of activation and relaxation, which for a 1 s contraction was calculated to be \( \approx 37\% \) of the total ATP consumption. The number of contractions during the LOW condition was 40-50% higher than the number during the HIGH condition for each sprint (Fig. 8.6). Therefore, an increased energy cost for activation/relaxation of the muscles during the LOW condition may partially explain the higher fatigue index during each sprint and the similar pattern of decline in power between sprints in the two conditions, despite the fact that the external mean power was lower in the LOW compared with the HIGH condition.

In summary, this study has shown that although the mean power output for a 6 s sprint is increased as the resistive load increases, the peak power output is relatively insensitive to changes in load in the range of 50 to 100 g·kg\(^{-1}\) body mass. During repeated sprints performed against HIGH and LOW resistive loads, mean power outputs showed a parallel pattern of decline between successive sprints, in spite of the 15-20% higher mean power generated against HIGH loads. Furthermore, the fatigue index within each sprint was twice as high in the LOW condition, but peak power output was identical between corresponding sprints for the two conditions. The increased power output and lower fatigue index in the HIGH condition may be related to the average pedal speed which was closer to an optimum pedal speed (\( \approx 120 \) revs·min\(^{-1}\)) as calculated from the power-velocity relationship. On the other hand, the extremely high pedal speeds reached when sprinting against the LOW load may be too fast for effective power generation. The possibility of an increased reliance on fast twitch fibres at these pedal speeds may further contribute to the decrease in power output.
CHAPTER IX

GENERAL DISCUSSION

The aim of this chapter is to provide an overview and integrate the findings of the studies conducted for this thesis. The main findings of these studies are summarised as follows:

1. During a 30 s sprint on a cycle ergometer, approximately 44% of the total work was generated during the first 10 s of the sprint, while the remaining ≈31% and ≈25% was generated between 10-20 s and 20-30 s, respectively. This was true for both the 1st and 2nd sprint.

2. The high power output during the initial 10 s of both sprint 1 and sprint 2 was mainly supported by energy supply from PCr degradation (30-35%) and anaerobic glycolysis (42-49%). However, the contribution from PCr was diminished during the remaining 20 s of the sprint, due to the decreased PCr concentration, leaving glycolysis and aerobic metabolism as the main energy sources (Chapters V and VII).

3. During recovery from a maximal 30 s sprint, PCr was resynthesised following an exponential time course with a mean half time of 56.6±7.3 s. Restoration of peak power output parameters (such as PPO, maxSp, MPO6) occurred in parallel with PCr resynthesis, despite muscle pH remaining low throughout recovery (=6.7), and calculated [Pi] and [H2PO4−] being unchanged between 1.5 and 6 min of recovery. Nevertheless, the restoration of both PCr and peak sprint performance was incomplete (85% and 90% of control values, respectively) after 6 min of rest following a 30 s sprint (Chapter IV).

4. Low intensity exercise performed between two 30 s sprints ("active recovery") improved power output restoration only during the initial 10 s of sprint 2 (by ≈3%), while the ability to accelerate (as reflected in Wacc) was improved by ≈8%. Active recovery resulted in an 18% higher VO2 during the 2nd sprint, compared with passive recovery (Chapter VI).

5. The recovery of muscle metabolism and power output was also examined following a 10 s and a 20 s sprint. PCr resynthesis 2 min after sprint 1 was similar after the 10 s sprint and the 20 s sprint (86% and 76% of resting, n.s.), but muscle lactate was different (38 vs 66 mmol·kg dry
Chapter IX: General Discussion

Muscle-I, respectively). Restoration of peak power output was complete when sprint 1 was .10 s, in spite of elevated muscle lactate and [Pi]. However, the mean power during the first 10 s of sprint 2 was 5% lower compared with sprint 1. Power output during sprint 2 after the 20 s sprint was lower compared with both sprint 1 (in the same experimental condition) and with sprint 2 when sprint 1 was 10 s (Chapter VII).

6: The significance of aerobic metabolism during repeated sprint exercise was indicated in two ways: (i) the percentage contribution of aerobic metabolism was as high as 43% of the total estimated energy utilisation (aerobic+anaerobic) during sprint 2. This percentage was increased to 65% during the last 20 s of the sprint, and aerobic metabolism sustained the high power output at a time when PCR was almost depleted and anaerobic glycolysis was considerably reduced, (ii) both PCR resynthesis and power output recovery during sprint 2 showed high correlations with %4M, which was taken as an index of aerobic fitness (r=0.82 to 0.94, P<0.01; Chapter V).

7. The effects of power-velocity relationship on power output during repeated sprints were assessed by sprinting against different resistive loads. A higher mean power was generated when sprinting against a heavy, compared with a light resistive load (mean pedal speed: =125 revs·min⁻¹ vs =175 revs·min⁻¹, respectively). During repeated 6 s sprints, the increased work output (heavy load) was not accompanied by a greater fatigue, and this was interpreted as an increased efficiency due to cycling closer to an "optimum pedal speed" of =120 revs·min⁻¹ (Chapter VIII).

The following discussion attempts to explain the above findings and propose possible mechanisms for the recovery of muscle metabolism and power output during repeated sprint exercise.

9.1. Power output and muscle metabolism during sprint 1

By combining the data from the studies described in Chapters IV, V and VII, the time course of changes in muscle metabolites during sprint 1 can be estimated. Thus, the first sprint has been divided into three 10 s blocks, and the corresponding mean power output during each 10 s block has been calculated. The muscle metabolite and performance data from the study in Chapter VII have been used for the first two blocks (0-10 s and 10-20 s), while results for the third 10 s block have been calculated using the combined 30 s data from Chapter IV and V (Fig. 9.1).
Fig. 9.1 Calculated anaerobic ATP utilisation rates (left) and mean power output (MPO; right) during the first sprint. Mean values are given for the time periods between 0-10 s, 10-20 s, 20-30 s.

Due to the training status of the subjects (moderately to well trained recreational athletes), the power output in all the studies was the highest reported using similar methods of measurement (e.g. Gaitanos et al., 1993; Lakomy, 1988). This high power output was also reflected in the muscle metabolite data, where the calculated ATP utilisation rate and lactate accumulation were higher than most published results (e.g. Boobis et al., 1987; Jones et al., 1985; McCartney et al., 1986).

An interesting observation from Fig. 9.1 and all power profiles presented in this thesis, was that power output during a 30 s sprint declined faster at the initial stages of the sprint and slower towards the end. This pattern was similar to the decreases in the calculated ATP utilisation rate (Fig. 9.1), even when aerobic metabolism was taken into account (Chapter VII). In a simplified scenario, the decrease in total (aerobic+anaerobic) ATP utilisation should match the decline in power output. However, this is hardly ever the case, especially when power output is generated using multi-joint movements. A combination of "metabolic" and "non-metabolic" (i.e. mechanical) factors may influence this relationship. The
following points raise some of the problems when attempting to relate changes in power output and ATP utilisation during sprint cycling:

- The working muscle mass is unknown, and it may be that additional and/or different (slow vs fast twitch) muscles and/or motor units are recruited at different stages of the sprint (i.e. when fatigue and slowing of the pedalling speed are prominent; Freund, 1983).
- The aerobic contribution to the sprint has been estimated from pulmonary gas exchange and was based on a series of assumptions (Chapter V).
- The “energy cost” of muscle contraction has been shown to be different for fast and slow twitch fibres. Electrical stimulation studies have also demonstrated an increase in “energy cost” when the frequency of contractions increased, despite the total contraction time and stimulation pulses remaining unchanged (Chasiotis et al., 1987; Bergstrom and Hultman, 1988).
- The notion of changes in efficiency as a result of the muscle power-velocity relationship when contraction speed changes, has been examined in animal muscle in situ (Haan et al., 1989; Lodder et al., 1991), but mainly power-velocity (and not energy cost) data have been reported for humans performing sprint exercise (Sargeant et al., 1981; McCartney et al., 1983b). Using theoretical calculations and power output data Lakomy (1988) suggested that a large part of fatigue during the acceleration phase of sprinting on a friction loaded ergometer, is mainly due to the power-velocity relationship and less a result of metabolic changes.
- During multi-joint alternating movements such as sprint cycling, external power is an expression of the combined action of many muscles including bi-articular muscles (which cross 2 or more joints). A characteristic pattern of muscle activation during the “push-down” phase of each pedal stroke is the co-contraction of knee extensors (quadriceps femoris) and knee flexors (biceps femoris, semimembranosus and semitendinosus) which also extend the hip (Jorge and Hull, 1986; Gregor et al., 1991). It has been proposed that this “so-called” Lombard’s paradox is involved in transfer of energy between joints (Ingen Schenau et al., 1990) and constitutes a further complication to the problem of matching muscle metabolite and external power output data during cycling. Moreover, when pedalling as fast as >170 revs-min⁻¹ it is possible that a significant amount of
energy will be "lost" in order to counteract inertial forces of the lower limbs which must be decelerated and re-accelerated in alternate directions for every pedal cycle (Kaneko and Yamazaki, 1978). This energy will be less when pedalling slower towards the end of the sprint.

The above points show that a one-to-one relationship is not necessarily expected when relating external power output measured during sprint cycling and changes in muscle metabolism, measured in the vastus lateralis muscle. Despite all these "confounding" factors, a fairly good agreement was observed between decrease in total ATP utilisation and decrease in power output from the first 10 s to the second 10 s of sprint 1 (40% vs 28%). It must be mentioned that mean pedal speed was \(\approx 149\) revs·min\(^{-1}\) (0-10 s) and was decreased to 120 revs·min\(^{-1}\) (10-20 s).

Although the greatest proportion of the metabolic changes occurred during the first 10 s of sprint 1, with a marked decrease in \([\text{PCr}]\) and \([\text{ATP}]\) and increase in muscle lactate, it is difficult to separate the individual contribution of each process to fatigue. This may be assessed better in a situation when muscle lactate and \([\text{H}^+]\) are high and constant, while \([\text{PCr}]\) varies, as seen during short-term recovery from a 30 s sprint (Chapter IV). Furthermore, comparisons between two 30 s sprints reduce the effects of the "non-metabolic" (mechanical) factors. This is because the range of pedal speeds attained and the time-course of changes in pedal speed are much closer when comparing two 30 s sprints, than when comparing the initial and final seconds within a single sprint.

9.2. \(\text{PCr resynthesis after sprint exercise}\)

One of the main findings in this thesis was that \(\text{PCr}\) was resynthesised following a single exponential time course, but failed to reach the resting levels even after 6 min of recovery. Most importantly, peak power parameters (PPO, maxSp, MPO\(_6\)) were restored in parallel with \(\text{PCr}\) despite the low muscle pH.

The rate of \(\text{PCr}\) resynthesis after exhausting dynamic (8.7 min) and isometric (45-55 s) exercise in man has been examined by Harris et al. (1976) using the biopsy technique. The time course of \(\text{PCr}\) resynthesis in
their study was found to be biphasic, and the half time for the fast component was \( \approx 22 \) s (Harris et al., 1976). Using the non-invasive \( ^{31}\text{P}-\text{MRS} \) technique, several studies have reported similar values for the half time of \( \text{PCr} \) resynthesis after submaximal contractions (Kushmerick and Meyer, 1985; McCully et al., 1991, 1993, 1994; Blei et al., 1993; Yoshida and Watari, 1993a).

Comparing the data from the study in Chapter IV with that of Harris et al., (1976) shows that following sprint exercise \( \text{PCr} \) resynthesis was slower than after dynamic exercise which exhausted the subjects in \( \approx 8.7 \) min (Fig. 9.2). In an attempt to explain that difference, the factors influencing \( \text{PCr} \) resynthesis must be considered (Harris et al., 1976; Sahlin et al., 1979; Chance et al., 1985; Sapega et al., 1987; Blei et al., 1993). These include:

1. Oxygen supply to the mitochondria, because the ATP for \( \text{PCr} \) resynthesis comes from oxidative phosphorylation.
2. Removal of \( \text{H}^+ \) from the muscle, because \([\text{H}^+]\) may influence the creatine kinase equilibrium.

Fig. 9.2. Time course of phosphocreatine (\( \text{PCr} \)) resynthesis in the present and other selected studies after exhausting exercise.
As suggested in Chapter VI, and supported by data from patients with vascular disease (Radda et al., 1982), muscle blood flow is the common regulator of O₂ supply and [H⁺] removal from the muscle (Fig. 9.3.). Therefore, there may be a lower O₂ supply and/or a slower H⁺ removal (and ultimately blood flow) after sprinting compared with cycling to exhaustion for ≈8.7 min. Interestingly, muscle lactate and pH data would suggest that no major differences exist in lactate and pH recovery between sprinting (Chapter IV) and cycling to exhaustion for 6-11 min (Sahlin et al., 1976). It is possible then that in this case, oxygen supply is the limiting factor.

The importance of oxygen supply compared with H⁺ efflux during recovery is implicit in the data of Harris et al., (1976). Two apparently conflicting features are seen when comparing metabolic recovery after dynamic and isometric exercise: PCr was resynthesised faster after dynamic exercise (see Fig. 9.2), but muscle lactate disappearance was faster after
isometric knee extension to fatigue (45-55 s). It must be noted that muscle lactate at the start of recovery was similar in the two modes of exercise (Harris et al., 1976; Sahlin et al., 1975, 1979; Sahlin and Ren, 1989).

Marlin (1989) provided an interesting explanation for the differences in muscle lactate disappearance after isometric and dynamic exercise, based on muscle-to-blood concentration ratios for lactate and [H+]$. A high ratio (i.e. high muscle and low blood lactate and [H$^+$]) is thought to promote lactate efflux from muscle to blood (Gladden, 1989; Renaud, 1989). This is probably the case after isometric knee extension where changes in blood metabolites are minimal, and therefore the blood perfusing the knee extensors during recovery has low lactate and [H$^+$]. On the other hand, greater cardiovascular responses (e.g. heart rate 182-204 beats·min$^{-1}$; Sahlin et al., 1976) and presumably muscle blood flow to the quadriceps are expected after the dynamic exercise (cycling for $\approx 9$ min). However, the blood perfusing the recovering muscle has high lactate and [H$^+$], and this may explain the slow lactate and H$^+$ efflux from the muscle (Gladden, 1989; Renaud, 1989). Nevertheless, a higher blood flow would also mean that more oxygen is delivered to the muscle, and may be related to the higher PCr resynthesis after dynamic exercise (Table 9.1).

Table 9.1. Comparisons between blood flow and metabolic recovery following isometric knee extension (66% maximal force, for 45-55 s), cycling to exhaustion ($\approx 8.7$ min) and 30 s of sprinting. Relative changes were estimated from the references quoted in the text and studies in this thesis. Crosses do not have any quantitative value, but are used to express relative differences between the three modes of exercise.

<table>
<thead>
<tr>
<th></th>
<th>Isometric</th>
<th>Dynamic</th>
<th>Sprinting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>muscle La/[$H^+$] efflux</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>muscle-to-blood La/$H^+$ gradient</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PCr resynthesis</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

La$^-$, lactate; PCr, phosphocreatine

The above arguments give support to the suggestion that there may be a reduced blood flow to the recovering muscles after sprinting, which poses a limitation to PCr resynthesis, making it similar to that seen after isometric exercise (Fig. 9.2). A decrease in blood pressure and an increase
in the intramuscular pressure (resulting from the large plasma volume shifts) in combination with the passive recovery (lack of "muscle pump" action) may be contributing factors to a reduced "local" muscle blood flow and PCr resynthesis during recovery (Sjøgaard, 1987, 1990). Therefore, the active recovery performed in the study described in Chapter VI may have resulted in an increased blood flow and PCr resynthesis, which would explain the improvement only during the first 10 s of sprint 2. Differences in PCr resynthesis as a result of different exercise intensities may explain why a complete restoration of peak power has been observed following only 1 min of rest after 2-6 min of cycling at 90% VO$_2$max, (Sargeant and Dolan, 1987; Hitchcock, 1989), but not even after 6 min of rest following a 30 s sprint (Chapter IV).

An important aspect of recovery from sprint exercise concerns aerobic metabolism. Both PCr resynthesis before the second sprint and a high contribution to energy supply by aerobic metabolism were related to endurance fitness and VO$_2$max. There is growing evidence that PCr resynthesis is faster in endurance trained athletes (Yoshida and Watari, 1993b), and it can be improved by endurance training (McCully et al., 1991). The significant contribution of aerobic metabolism, especially towards the end of the sprint, provides valuable energy at times when the other energy systems fail due to lack of substrate (PCr) or due to enzyme inhibition (glycolysis).

Taken collectively, the studies presented in this thesis have provided evidence to support the notion that muscle can produce high power outputs under acidotic conditions (muscle pH = 6.7). The fact that power output during the initial seconds of sprint 2 was related to PCr content before the sprint, highlights the importance of PCr. This does not imply that the other energy systems are of little importance, since a large part of energy is not supplied by PCr. The significance of PCr lies in the fact that it can be resynthesised quickly and therefore is a readily available energy source. Furthermore, the PCr system is the fastest to respond to the ATPase activity and to buffer the products of ATP hydrolysis (Funk et al., 1989). The following section attempts to provide a framework in order to explain the relationship of the recovery in power and metabolism at the level of the contracting fibre.
CHAPTER IX: General Discussion

9.3. Metabolic basis of fatigue and recovery during repeated sprinting

Although changes in muscle metabolites during sprint 2 can explain the major part of the decrease in power output and have provided useful information about the relative importance of the energy systems during a repeated sprint, there is still uncertainty concerning the exact mechanisms which regulate fluxes in the metabolic pathways.

During sprint exercise ATP must be regenerated at rapid rates if power output is to be maintained. As shown in this thesis, and also reported by other authors (Jones et al., 1985; Gaitanos et al., 1993), both PCr and anaerobic glycolysis are the main sources of ADP rephosphorylation during the start of the sprint, but their contribution is progressively decreased, while oxidative phosphorylation becomes increasingly important. Following short term recovery, PCr is resynthesised (although it does not reach resting levels), and can be broken down at the same rate as that measured during the first sprint (Chapters V and VII). However, the ADP rephosphorylation via anaerobic glycolysis can not match sprint 1 values (see Fig. 5.9 and 7.5), possibly as a result of the low muscle pH (Trivedi and Danforth, 1966; Spriet et al., 1987b). In order for power output to be reproduced during sprint 2, ATP must be regenerated at the same rate as in sprint 1 (e.g. =15 mmol·kg dry muscle$^{-1}$·s$^{-1}$ in the first 6 s; Gaitanos et al., 1993). If one of the ADP rephosphorylation mechanisms fails to function at the same rate as in sprint 1, then power (which is itself the rate of work done) is expected to decrease. This explanation, i.e. that the inability to regenerate ATP at high rates results in fatigue, has been proposed in the past for exercise demanding lower ATP turnover rates (Katz et al., 1986a; Sahlin, 1986a, 1992; Sahlin and Ren, 1989).

However, this theory has been disputed because the total ATP measured in whole muscle never drops below 50% of the resting value (Boobis et al., 1983; Harris et al., 1991; Sewell et al., 1992). Thus, concentrations of ATP are well above the Km of myosin ATPase for ATP which is close to 1 mmol·kg dry muscle$^{-1}$ (Glyn and Sleep, 1985). The decrease of ATP in the present study was 25-30%, and the largest part of the decrease occurred within the first 10 s of sprint 1. The small decrease of ATP during sprint 2 was also confined to the first 10 s. The question, therefore arises: how can
fatigue due to an insufficient rate of energy supply be reconciled with the relatively high levels of ATP, i.e. if there is an energy crisis in the cell, why is ATP concentration maintained?

Unfortunately, there is no simple answer to these questions. Some insight into the problem has been gained by analysing the metabolite contents of single fibres. Harris (1985) has proposed that ATP depletion in a small percentage of fibres (especially the fast twitch) may result in a failure to recruit them during exercise, and therefore a reduced power output. More recently, Soderlund and Hultman (1990) have shown that after intermittent electrical stimulation for 83 s with occluded blood flow, force generation decreased to 22% of the initial value, while in 11% of the fibres the ATP content was < 10 mmol·kg dry muscle⁻¹. This evidence suggests that measurements of ATP contents in “mixed” muscle may be misleading, as ATP may fall considerably in single fibres. This area deserves further investigation.

Alternatively, it has been proposed that ATP within the cell may be compartmentalised (Bessman and Geiger, 1981; Wallimann et al., 1992). This attractive suggestion would explain why fatigue occurs without a total depletion of ATP, since it is the ATP near the contractile mechanism that may be depleted. Although the notion of a “compartment” as a separate region has been disputed (Meyer et al., 1984), the existence of so-called “functional compartments” has been shown in several studies (Rossi et al., 1990; Kingsley et al., 1991; Wallimann et al., 1992; Korge and Campbell, 1994). According to that concept there is a functional coupling between ATP-requiring processes and ATP-regenerating processes at the site of ATP utilisation. For example, the MM (muscle) creatine kinase isoform has been found to be localised on the M-band of the sarcomere so that the products of the myosin ATPase are removed immediately and ATP is regenerated (Wallimann et al., 1992). Instead of looking at “global” levels of metabolites, attention has been focused on the close proximity of the ATPases. An increase in the rate of ATPase-catalysed ATP hydrolysis (increased energy demand) will tend to create a microenvironment in the vicinity of the enzyme with an elevated [ADP], [Pi], [H⁺], and depressed [ATP]. If the system responsible for local ATP regeneration (e.g. creatine kinase or glycolysis) fails to remove the products of the reaction, then the reaction will slow down (Kentish, 1986; Cooke et al., 1988; Cooke and Pate, 1990).
The ATP utilising processes involved in muscular contraction are catalysed by myosin ATPase, Ca$^{2+}$ transporting ATPase, and Na$^+$-K$^+$ ATPase (Fig. 9.4). The release of Ca$^{2+}$ from the sarcoplasmic reticulum and the formation of cross bridges are processes that do not require any ATP. However, muscle relaxation requires ATP and also removal of ADP, Pi and H$^+$ for (i) calcium reuptake by the calcium pump, and (ii) detachment of cross bridges (Edwards et al., 1975; Dawson et al., 1980; Jones and Round, 1990). Therefore, it follows that an impaired function of myosin and Ca$^{2+}$ transporting ATPase will affect the speed of contraction. Furthermore, a decreased function of Na$^+$-K$^+$ ATPase will affect ion balance, and will eventually disturb the excitation-contraction coupling. It has been suggested that repeated action potentials may lead to Na$^+$ deficiency and
K\textsuperscript{+} accumulation in the T-tubules, which will both impair action potential conduction (Sjogaard, 1990; Westerblad \textit{et al.}, 1991; Allen \textit{et al.}, 1992).

For power generation (which involves both force and velocity), the function of all the ATPases is important. The discovery of functionally coupled processes in muscle cells for all three ATPases, has provided a new insight into the causes of fatigue (for review see Wallimann \textit{et al.}, 1992). Several studies have shown that creatine kinase isoenzymes are localised at the myofibrillar M-band, the sarcoplasmic reticulum and the sarcolemma membrane, and are important regulators of relaxation and tension development by preventing product accumulation and providing ATP in the immediate vicinity of each ATPase (Baskin and Deamer, 1970; Ventura-Clapier \textit{et al.}, 1987; Hoerter \textit{et al.}, 1988; Rossi \textit{et al.}, 1990; Wallimann \textit{et al.}, 1992; Korge and Campbell, 1994).

Much of the indirect evidence which has led to the proposed concept of adenine nucleotide compartmentalisation has come from experiments with the ischemic heart, where it was found that contracture (rigor) was more closely related to glycolytic ATP production than to total cellular ATP content, indicating that functionally important regional differences in [ATP] within the cells are likely to occur (Kingsley \textit{et al.}, 1991).

Taken together, this evidence may provide an explanation for numerous findings that changes in skeletal muscle contractility do not correlate with changes in tissue ATP during fatigue. Therefore, it follows that metabolite levels in the microenvironment of ATPases are crucial for regulating muscle function. In order to appreciate the importance of energy regeneration systems, they must be viewed not only as ATP providers but also as pathways for removal/utilisation of the products of ATP hydrolysis. In this sense glycolytic enzymes bound to contractile proteins (Roy \textit{et al.}, 1991) and creatine kinase bound to the M-band of the sarcomere (Wallimann \textit{et al.}, 1992) work in concert to maintain optimum conditions for the ATPase. However, PCr may be of greater importance when the system is maximally and repeatedly stressed. As seen in this thesis, the glycolytic rate was significantly reduced during a repeated sprint. Furthermore, there is evidence to suggest that glycolysis may be totally inhibited after a series of ten short sprints (Gaitanos \textit{et al.}, 1993). However, in all cases PCr is resynthesised during recovery and is readily available for rapid use during the first seconds of a repeated sprint.
9.4. Conclusion

The studies in this thesis have provided evidence to support the suggestion that the ability to reproduce high power output during the initial seconds of repeated sprint exercise is related to the availability of PCr. Therefore, its resynthesis during recovery is important. It has been suggested that blood flow to the previously exercising muscles may impose a limitation to PCr and power output recovery. Further research is needed to elucidate the role of local circulation in the recovery process in combination with metabolite changes at the single fibre level. The significance of aerobic metabolism for repeated sprint exercise was a major finding in this thesis, not only for its contribution to energy supply but also for its relationship with metabolic recovery. Finally, it was shown that measurement of power output during sprint cycling is affected by the power-velocity relationship of the muscles involved. Certainly, more problems of interpretation arise when a friction-loaded ergometer is used and the pedal speed varies during a sprint (as opposed to an isokinetic ergometer). However, the features of acceleration and deceleration (as fatigue occurs) are inherent with human movement, and therefore a part of the fatigue process.

There are still many questions to be answered concerning the complex phenomena of fatigue and recovery during sprint exercise.
REFERENCES


References


References


References


APPENDICES

APPENDIX A ......................................................... Blood Metabolite Assays
APPENDIX B ........................................................... Muscle Metabolite Assays
APPENDIX C ......................................................... Spectrophotometric Muscle Metabolite Assays
APPENDIX D ......................................................... Buffers and reagents for muscle metabolite assays
APPENDIX E ........................................................... Calculations, Conversions and Formulae
APPENDIX F ........................................................... Modelling Phosphocreatine Resynthesis
APPENDIX G ......................................................... Respiratory parameters during recovery from a 30 s sprint
APPENDIX A: Blood metabolite assays

APPENDIX A

BLOOD METABOLITE ASSAYS

Fluorimetric assay for the determination of blood lactate (modified from Maughan, 1982)

Principle: $\text{NAD}^+ \rightarrow \text{NADH} + H^+$

\[
\text{Lactate} \xrightarrow{\text{lactate dehydrogenase}} \text{Pyruvate}
\]

Reagents:
- Buffer: Hydrazine 1.1 mol·l$^{-1}$, pH 9.0 with 1 mmol·l$^{-1}$ EDTA·Na$_2$
- Cofactor: NAD
- Enzyme: lactate dehydrogenase (LDH) 5500 U·ml$^{-1}$ (undiluted)
- Standard: L-Lactate 1 mol·l$^{-1}$ (stock solution)
- Diluent: 0.07 mol·l$^{-1}$ HCl

Stock standards were prepared before each study and stored at -20°C:

<table>
<thead>
<tr>
<th>L-Lactate 1 mol·l$^{-1}$ (μl)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>50</th>
<th>80</th>
<th>100</th>
<th>120</th>
<th>150</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 mol·l$^{-1}$ perchloric acid (ml)</td>
<td>10</td>
<td>9.98</td>
<td>9.96</td>
<td>9.95</td>
<td>9.92</td>
<td>9.90</td>
<td>9.88</td>
<td>9.85</td>
<td>9.80</td>
</tr>
<tr>
<td>Lactate concentration (mmol·l$^{-1}$)</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

Working standards were prepared by diluting 20 μl of each of the above standards into 200 μl of 0.4 mol·l$^{-1}$ (~2.5%) perchloric acid.

Reaction mixture (final concentration):
- Buffer 1 ml
- NAD 2 mg (2.98 mmol·l$^{-1}$)
- LDH 10 μl (54.46 U·ml$^{-1}$)

Procedure:
1. 200 μl of reaction mixture was added to 20 μl aliquots of duplicate samples, perchloric acid blanks and standards (mix well).
2. After 30 min incubation at room temperature, 1 ml of diluent (0.07 mol·l$^{-1}$ HCl) was added, the contents were mixed and fluorescence was read. Lactate concentration was calculated from the standard curve.
APPENDIX A: Blood metabolite assays

Spectrophotometric determination of plasma ammonia (340 nm)

Principle:

\[
\text{NADPH} \rightarrow \text{NADP}^+ \\
\text{a-oxoglutarate} + \text{NH}_4^+ \xrightarrow{\text{glutamate dehydrogenase}} \text{L-glutamate} + \text{H}_2\text{O}
\]

Reagents: The Boehringer Mannheim MPR 1 Ammonia kit was used (Cat. No. 125 857)

Initial concentrations of solutions:

<table>
<thead>
<tr>
<th>Reagent solution:</th>
<th>NADPH 0.10 mmol·l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>triethanolamine buffer 0.15 mol·l⁻¹, pH 8.6</td>
<td></td>
</tr>
<tr>
<td>a-oxoglutarate 15 mmol·l⁻¹</td>
<td></td>
</tr>
<tr>
<td>ADP 1.5 mmol·l⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

Enzyme: Glutamate dehydrogenase (GLDH) ≥ 755 U·ml⁻¹

Procedure:

1. 500 μl of the reagent solution was added to 100 μl aliquots of samples or standards* in a 1 ml disposable cuvette (light path: 1 cm). 2-4 reagent blanks were used for every assay (600 μl of the reagent solution in each cuvette).

2. The contents of each cuvette were mixed well upon addition of the reagent solution and were incubated at room temperature for 10 min.

3. After the 10 min of incubation the initial absorbance (A₁) of samples, blanks and standards was read at 340 nm (N.B. absorbance decrease).

4. After the absorbance was read, 4 μl of enzyme (GLDH) was added to each cuvette with a positive displacement pipette, the contents of each cuvette were mixed well and incubated for 10 min.

5. After the 10 min of incubation the absorbance of samples, blanks and standards was read again (A₂).

6. Steps 4 and 5 were repeated, and a final absorbance of samples, blanks and standards was read (A₃).

*To control accuracy and precision of ammonia determinations, a set of 3 standards (58.8 μmol·l⁻¹, 117.6 μmol·l⁻¹ and 176.5 μmol·l⁻¹) was used (Preciset® Ammonia, Cat. No. 166 570)
Cuvettes were sealed during incubations with a plastic cap. If the ammonia concentration exceeded 412 µmol·l⁻¹, plasma was diluted (1:2) with double distilled water and assay was repeated. The concentration of ammonia in the sample was calculated using the extinction coefficient for NADPH, as follows:

\[(A_1 - A_2) - (A_2 - A_3) = \Delta A_{\text{BLANK}} \text{ or } \Delta A_{\text{SAMPLE/STANDARD}}\]

Concentration (µmol·l⁻¹): \[959 \times (\Delta A_{\text{SAMPLE/STANDARD}} - \Delta A_{\text{BLANK}})\]

Standards were used only to check the assay.
APPENDIX B

MUSCLE METABOLITE ASSAYS

Adenosine triphosphate (ATP) and Phosphocreatine (PCr)

Principle:

\[
P\text{-Creatine} + \text{ADP} \xrightarrow{\text{creatine kinase}} \text{Creatine} + \text{ATP} \\
\text{ATP} + \text{glucose} \xrightarrow{\text{hexokinase}} \text{ADP} + \text{glucose-6-P} \\
\text{NADP}^{+} \xrightarrow{\text{glucose-6-P dehydrogenase}} \text{NADPH} + \text{H}^{+} \\
\text{Glucose-6-P} \xrightarrow{\text{6-P-gluconolactone}} \text{6-P-gluconolactone}
\]

Buffer: Tris-HCl 50 mmol·l⁻¹, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)
Cofactor: NADP 5 mmol·l⁻¹
Enzymes*: G6P-DH 14 U·ml⁻¹; HK 28 U·ml⁻¹; CK 1260 U·ml⁻¹
Reagents: ADP 10 mmol·l⁻¹; Glucose 10 mmol·l⁻¹; Dithiothreitol (DTT) 50 mmol·l⁻¹; MgCl₂ 100 mmol·l⁻¹
Standards: ATP 2 mmol·l⁻¹; PCr 2 mmol·l⁻¹
Diluent: Carbonate buffer 20 mmol·l⁻¹, pH 10.0

Working standards were prepared daily as follows:

<table>
<thead>
<tr>
<th>ATP 2 mmol·l⁻¹ (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>double distilled water (µl)</td>
</tr>
<tr>
<td>2000</td>
</tr>
<tr>
<td>ATP concentration (µmol·l⁻¹)</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>PCr concentration (µmol·l⁻¹)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Reaction mixture was prepared immediately prior to analysis for 3 sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

* enzymes were diluted using Tris-HCl 20 mmol·l⁻¹, pH 8.1 with 0.02% BSA
G6P-DH, glucose-6-P dehydrogenase; HK, hexokinase; CK, creatine kinase
APPENDIX B: Muscle metabolite assays

Reaction mixture (final concentrations):

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>1 ml</td>
</tr>
<tr>
<td>NADP</td>
<td>10 µl (0.046 mmol·l⁻¹)</td>
</tr>
<tr>
<td>ADP</td>
<td>10 µl (0.091 mmol·l⁻¹)</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 µl (0.091 mmol·l⁻¹)</td>
</tr>
<tr>
<td>DTT</td>
<td>10 µl (0.457 mmol·l⁻¹)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>50 µl (4.566 mmol·l⁻¹)</td>
</tr>
<tr>
<td>G6P-DH</td>
<td>5 µl (0.064 U·ml⁻¹)</td>
</tr>
</tbody>
</table>

Procedure:

1. 20 µl of extract was pipetted into a fluorimeter tube and diluted with 100 µl of double-distilled water (1:6 dilution). 10 µl aliquots of the diluted extract were pipetted into 6 fluorimeter tubes resulting in 3 sets of duplicate samples. Three sets of double distilled water blanks and 1 set of standards were also pipetted (10 µl aliquots).

2. 200 µl of the above reaction mixture was added to one set of tubes and blanks (G6P determination; STEP 1).

3. 5 µl of HK (0.127 U·ml⁻¹) was added per ml of remaining reaction mixture and 200 µl of this reaction mixture was then added to the second set of tubes, blanks and ATP standards (ATP+G6P determination; STEP 2).

4. 10 µl of CK (11.351 U·ml⁻¹) was added per ml of remaining reaction mixture and 200 µl of this reaction mixture was then added to the third set of tubes and blanks (ATP+G6P+PCr determination; STEP 3). Furthermore, 1 ml of this reaction mixture was added to 50 µl of 200 µmol·l⁻¹ PCr standard and the reaction was followed in the fluorimeter and recorded on a chart recorder, to ensure that the reaction had reached completion.

5. After incubating for 30-40 min at room temperature (reaction completed), 1 ml of carbonate buffer was added to each tube using a Hamilton automatic dispenser (Hamilton microlab 1000; Switzerland), and after thorough mixing fluorescence was read.

For ATP determination: The relative fluorescence of G6P (STEP 1) was subtracted from the relative fluorescence of ATP+G6P (STEP 2).

For PCr determination: The relative fluorescence of ATP+G6P (STEP 2) was subtracted from the relative fluorescence of ATP+G6P+PCr (STEP 3). The concentrations of ATP and PCr were determined using simple linear regression analysis of the standard concentrations (ATP) on fluorescence readings. Concentrations were corrected for dilutions during the extraction procedure and the 1:6 dilution during this assay:

Concentration (mmol·kg dry muscle⁻¹): regression value x 0.125 x 6

*relative fluorescence = (sample fluorescence - respective blank fluorescence)*
Adenosine diphosphate (ADP), adenosine monophosphate (AMP) and pyruvate

Principle:

\[ \text{AMP} + \text{ATP} \xrightarrow{\text{adenylate kinase}} 2 \text{ADP} \]

\[ \text{ADP} + P\text{-pyruvate} \xrightarrow{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate} \]

\[ \text{Pyruvate} \xrightarrow{\text{lactate dehydrogenase}} \text{lactate} \]

Buffer: Imidazole-HCl 50 mmol·l\(^{-1}\), pH 7.0
Cofactor: NADH (grade I) 1 mmol·l\(^{-1}\)
Enzymes*: AK 35 U·ml\(^{-1}\); PK 75 U·ml\(^{-1}\); LDH 40 U·ml\(^{-1}\)
Reagents: ATP 0.5 mmol·l\(^{-1}\); phosphoenol pyruvate (PEP) 2 mmol·l\(^{-1}\); MgCl\(_2\) 100 mmol·l\(^{-1}\); KCl 3 mol·l\(^{-1}\);
edthylendiamine tetra-acetic disodium salt (EDTA:Na\(_2\)) 100 mmol·l\(^{-1}\)
Standards: ADP 2 mmol·l\(^{-1}\)
Diluent: Carbonate buffer 20 mmol·l\(^{-1}\), pH 10.0

Working standards were prepared daily as follows:

<table>
<thead>
<tr>
<th>ADP 2 mmol·l(^{-1}) (µl)</th>
<th>0</th>
<th>10</th>
<th>30</th>
<th>50</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP concentration (µmol·l(^{-1}))</td>
<td>0</td>
<td>10</td>
<td>30</td>
<td>50</td>
<td>80</td>
</tr>
</tbody>
</table>

Reaction mixture was prepared immediately prior to analysis for 3 sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

* enzymes were diluted using Tris-HCl 20 mmol·l\(^{-1}\), pH 8.1 with 0.02% BSA
AK, adenylate kinase; PK, pyruvate kinase; LDH, lactate dehydrogenase
APPENDIX B: Muscle metabolite assays

Reaction mixture (final concentrations):

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>1 ml</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>20 µl (1.874 mmol·l⁻¹)</td>
</tr>
<tr>
<td>KCl</td>
<td>25 µl (70.291 mmol·l⁻¹)</td>
</tr>
<tr>
<td>ATP</td>
<td>10 µl (5 µmol·l⁻¹)</td>
</tr>
<tr>
<td>NADH</td>
<td>5 µl (5 µmol·l⁻¹)</td>
</tr>
<tr>
<td>PEP</td>
<td>5 µl (9 µmol·l⁻¹)</td>
</tr>
<tr>
<td>EDTA·Na₂</td>
<td>2 µl (0.187 mmol·l⁻¹)</td>
</tr>
</tbody>
</table>

Procedure:

1. 10 µl aliquots of undiluted extract were pipetted into 4 pairs of fluorimeter tubes resulting in 4 sets of duplicate samples. Four sets of double distilled water blanks and 1 set of standards were also pipetted (10 µl aliquots).
2. 200 µl of the above reaction mixture was added to one set of tubes and blanks (extract blanks; STEP 1).
3. 10 µl of LDH (0.371 U·ml⁻¹) was added per ml of remaining reaction mixture and 200 µl of this reaction mixture was then added to the second set of tubes and blanks (extract blanks + pyruvate determination; STEP 2).
4. 10 µl of PK (0.684 U·ml⁻¹) was added per ml of remaining reaction mixture and 200 µl of this reaction mixture was then added to the third set of tubes blanks and standards (extract blanks + pyruvate + ADP determination; STEP 3).
5. 10 µl of AK (0.322 U·ml⁻¹) was added per ml of remaining reaction mixture and 200 µl of this reaction mixture was then added to the fourth set of tubes and blanks (extract blanks + pyruvate + ADP + 2AMP determination; STEP 4).
6. After incubating for 30 min at room temperature (reaction completed), 1 ml of carbonate buffer was added to each tube using a Hamilton automatic dispenser, and after thorough mixing fluorescence was read. 

   For pyruvate determination: The relative fluorescence of (STEP 1) was subtracted from the relative fluorescence of STEP 2. However, a separate assay was used for pyruvate determination (see following pages).

   For ADP determination: The relative fluorescence of STEP 2 was subtracted from the relative fluorescence of STEP 3.

   For AMP determination: The relative fluorescence of STEP 3 was subtracted from the relative fluorescence of STEP 4.

The concentrations of ADP, 2AMP and pyruvate were determined using simple linear regression analysis of the standard concentrations (ADP) on fluorescence readings. Concentrations were corrected for the dilution during the extraction procedure:

Concentration (mmol·kg dry muscle⁻¹): regression value x 0.125

\[ \text{relative fluorescence} = (\text{respective blank fluorescence} - \text{sample fluorescence}) \]
APPENDIX B: Muscle metabolite assays

Creatine

Principle:

\[
\begin{align*}
\text{Creatine} + \text{ATP} & \xrightarrow{\text{creatine kinase}} \text{P-Creatine} + \text{ADP} \\
\text{ADP} + \text{P-pyruvate} & \xrightarrow{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate}
\end{align*}
\]

\[
\begin{align*}
\text{Pyruvate} & \xrightarrow{\text{lactate dehydrogenase}} \text{lactate}
\end{align*}
\]

Buffer: Imidazole-HCl 50 mmol·l⁻¹, pH 7.5
Cofactor: NADH (grade I) 1 mmol·l⁻¹
Enzymes: CK 1260 U·ml⁻¹; PK 75 U·ml⁻¹; LDH 240 U·ml⁻¹
Reagents: ATP 10 mmol·l⁻¹; phosphoenol pyruvate (PEP) 2 mmol·l⁻¹; MgCl₂ 100 mmol·l⁻¹; KCl 3 mol·l⁻¹; EDTA-Na₂ 100 mmol·l⁻¹.
Standards: Creatine 2 mmol·l⁻¹
Diluent: Carbonate buffer 20 mmol·l⁻¹, pH 10.0

Working standards were prepared daily as follows:

<table>
<thead>
<tr>
<th>Creatine 2 mmol·l⁻¹ (µl)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>double distilled water (µl)</td>
<td>2000</td>
<td>1950</td>
<td>1900</td>
<td>1850</td>
</tr>
<tr>
<td>Creatine concentration (µmol·l⁻¹)</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

Reaction mixture was prepared immediately prior to analysis for 2 sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

Reaction mixture (final concentrations):

<table>
<thead>
<tr>
<th>Buffer</th>
<th>1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂</td>
<td>50 µl (4.413 mmol·l⁻¹)</td>
</tr>
<tr>
<td>KCl</td>
<td>10 µl (26.478 mmol·l⁻¹)</td>
</tr>
<tr>
<td>ATP</td>
<td>20 µl (0.177 mmol·l⁻¹)</td>
</tr>
<tr>
<td>NADH</td>
<td>15 µl (13 µmol·l⁻¹)</td>
</tr>
<tr>
<td>PEP</td>
<td>25 µl (44 µmol·l⁻¹)</td>
</tr>
<tr>
<td>PEP</td>
<td>1 µl (88 µmol·l⁻¹)</td>
</tr>
<tr>
<td>LDH</td>
<td>2 µl (0.424 U·ml⁻¹)</td>
</tr>
<tr>
<td>PK</td>
<td>10 µl (0.662 U·ml⁻¹)</td>
</tr>
</tbody>
</table>

*enzymes were diluted using Tris·HCl 20 mmol·l⁻¹, pH 8.1 with 0.02% BSA
CK, creatine kinase; PK, pyruvate kinase; LDH, lactate dehydrogenase
APPENDIX B: Muscle metabolite assays

Procedure:

1. 20 μl of extract was pipetted into a fluorimeter tube and diluted with 100 μl of double-distilled water (1:6 dilution). 10 μl aliquots of the diluted extract were pipetted into 4 fluorimeter tubes resulting in 2 sets of duplicate samples. Two sets of double distilled water blanks and 1 set of standards were also pipetted (10 μl aliquots).

2. 200 μl of the above reaction mixture was added to one set of tubes and blanks (ADP+pyruvate determination; STEP 1).

3. 10 μl of CK (11.024 U·ml⁻¹) was added per ml of remaining reaction mixture and 200 μl of this reaction mixture was then added to the second set of tubes, blanks, and Creatine standards (Creatine+ADP+pyruvate determination; STEP 2). Furthermore, 1 ml of this reaction mixture was added to 50 μl of 150 μmol·l⁻¹ Creatine standard and the reaction was followed in the fluorimeter and recorded on a chart recorder, to ensure that the reaction had reached completion.

5. After incubating for 50-60 min at room temperature (reaction completed), 1 ml of carbonate buffer was added to each tube using a Hamilton automatic dispenser, and after thorough mixing fluorescence was read. For Creatine determination: The relative fluorescence\(^{\dagger}\) of ADP+pyruvate (STEP 1) was subtracted from the relative fluorescence of Creatine+ADP+pyruvate (STEP 2). The concentration of Creatine was determined using simple linear regression analysis of the standard concentrations (Creatine) on fluorescence readings. Concentrations were corrected for dilutions during the extraction procedure and the 1:6 dilution during this assay:

\[
\text{Concentration (mmol·kg dry muscle}^{-1}) = \text{regression value} \times 0.125 \times 6
\]

\(\dagger\) relative fluorescence = (respective blank fluorescence - sample fluorescence)
Glycogen

Glycogen was determined on both the acid precipitated muscle pellet (acid insoluble glycogen fraction) and the neutralised extract (acid soluble glycogen fraction) by measuring the glucosyl units obtained after acid (HCl) hydrolysis of glycogen (Jansson, 1981).

Total muscle glycogen was defined as the sum of these two fractions, minus the glucose and G6P present in the neutralised extract.

Acid Hydrolysis:

Reagents: HCl 1 mol·l⁻¹; NaOH 6 mol·l⁻¹

Procedure:
1. 100 µl of 1 mol·l⁻¹ HCl for each mg of muscle powder was added to the muscle pellet left in the bottom of each tube after the extraction procedure (in a screw-top eppendorf tube).
2. 100 µl of 1 mol·l⁻¹ HCl was also added to 20 µl of undiluted neutralised extract from the same sample (in a screw-top eppendorf tube).
3. The eppendorf tubes were tightly sealed and then were gently mixed and boiled for 2 hours in a water bath. The tubes were then centrifuged for 3 min and left at room temperature to cool down.

The acid hydrolysed extract only was neutralised with 15 µl of 6 mol·l⁻¹ NaOH.

Acid insoluble glycogen was assayed spectrophotometrically using a Glucose Test Combination (GOD/Perid method). Acid soluble glycogen was assayed fluorimetrically for the glucosyl units (glucose) produced.

Acid insoluble glycogen (hydrolysed muscle pellet)

Principle:

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{glucose oxidase}} \text{gluconate} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{ABTS} \xrightarrow{\text{horseradish peroxidase}} \text{coloured complex} + \text{H}_2\text{O}
\]

where:

ABTS = di-ammonium 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonate)
The intensity of the colour change is directly proportional to the concentration of glucose, and can be measured as differences in absorbance on a spectrophotometer. Therefore, the glucose concentration of a sample can be calculated by using the standard of known concentration provided with the analysis kit.

Reagents: GOD/Perid reagent containing:
- phosphate buffer 100 mmol·l⁻¹, pH 7.0
- horseradish peroxidase (POD) 0.8 U·ml⁻¹
- glucose oxidase (GOD) 10 U·ml⁻¹; ABTS 1.0 mg·ml⁻¹.
Reagent was stored in a dark bottle at 4°C.

Standard: Glucose 0.505 mmol·l⁻¹

Procedure:
1. 10μl aliquots from the hydrolysed supernatant (from the muscle pellet) were pipetted in triplicate into fluorimetric tubes. Five standards and five double-distilled water blanks were also pipetted (10μl aliquots).
2. 2 ml of GOD/Perid reagent were added to each cuvette using a Hamilton automatic dispenser.
3. Following 30 min of incubation at room temperature, the absorbance (A) of samples and standards was measured against the distilled water blanks at 436 nm.

Acid insoluble glycogen concentration (mmol glucosyl units·kg dry muscle⁻¹) was calculated as follows:

\[
\frac{A_{\text{sample}}}{A_{\text{standard}}} \times 50.5
\]
**APPENDIX B: Muscle metabolite assays**

**Acid soluble glycogen (hydrolysed muscle extract)**

**Principle:**

\[
\text{ATP + glucose} \xrightarrow{\text{hexokinase}} \text{ADP + glucose-6-P}
\]

\[
\text{NADP}^+ \xrightarrow{\text{NADPH} + \text{H}^+} \text{glucose-6-P dehydrogenase}
\]

Buffer: Tris-HCl 100 mmol·l⁻¹, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)

Cofactor: NADP 5 mmol·l⁻¹

Enzymes*: G6P-DH 7 U·ml⁻¹; HK 28 U·ml⁻¹

Reagents: ATP 200 mmol·l⁻¹; Dithiothreitol (DTT) 50 mmol·l⁻¹; MgCl₂ 100 mmol·l⁻¹; EDTA·Na₂ 100 mmol·l⁻¹

Standard: Glucose 0.505 mmol·l⁻¹

Diluent: Carbonate buffer 20 mmol·l⁻¹, pH 10.0

Working standards were prepared daily as follows:

| Glucose 0.505 mmol·l⁻¹ (μl) | 0 | 20 | 50 | 100 |
| double distilled water (μl) | 505 | 485 | 455 | 405 |
| Glucose concentration (μmol·l⁻¹) | 0 | 20 | 50 | 100 |

Reaction mixture was prepared immediately prior to analysis for 2 sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

**Reaction mixture (final concentrations):**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>1 ml</td>
</tr>
<tr>
<td>NADP</td>
<td>6 μl (29 μmol·l⁻¹)</td>
</tr>
<tr>
<td>ATP</td>
<td>1.5 μl (0.29 mmol·l⁻¹)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>10 μl (0.966 mmol·l⁻¹)</td>
</tr>
<tr>
<td>EDTA·Na₂</td>
<td>5 μl (0.483 mmol·l⁻¹)</td>
</tr>
<tr>
<td>DTT</td>
<td>10 μl (0.483 mmol·l⁻¹)</td>
</tr>
<tr>
<td>G6P-DH</td>
<td>3 μl (0.02 U·ml⁻¹)</td>
</tr>
</tbody>
</table>

* enzymes were diluted using Tris-HCl 20 mmol·l⁻¹, pH 8.1 with 0.02% BSA

G6P-DH, glucose-6-P dehydrogenase; HK, hexokinase
APPENDIX B: Muscle metabolite assays

Procedure:
1. 20 µl of neutralised hydrolysed extract was pipetted into 2 sets of duplicate samples. Two sets of double distilled water blanks and 1 set of standards were also pipetted (20 µl aliquots).
2. 200 µl of the above reaction mixture was added to one set of samples and blanks (G6P determination; STEP 1).
3. 5 µl of HK (0.135 U·ml⁻¹) was added per ml of remaining reaction mixture and 200 µl of this reaction mixture was then added to the second set of samples, blanks and Glucose standards (Glucose+G6P determination; STEP 2).
Furthermore, 1 ml of this reaction mixture was added to 50 µl of 100 µmol·l⁻¹ Glucose standard and the reaction was followed in the fluorimeter and recorded on a chart recorder, to ensure that the reaction had reached completion.
5. After incubating for 30 min at room temperature (reaction completed), 1 ml of carbonate buffer was added to each tube using a Hamilton automatic dispenser, and after thorough mixing fluorescence was read.

Acid soluble glycogen determination: The relative fluorescence of G6P (STEP 1) was subtracted from the relative fluorescence of Glucose+G6P (STEP 2).
The concentrations of Glycogen (glucosyl units) and G6P were determined using simple linear regression analysis of the standard concentrations (Glucose) on fluorescence readings. Concentrations were corrected for dilutions during the extraction procedure and acid hydrolysis.

Acid soluble glycogen concentration (mmol glucosyl units·kg dry muscle⁻¹) was calculated as follows: regression value x 0.125 x 6.75

Muscle free glucose, obtained from another assay, was subtracted from the results to give the true acid-soluble glycogen concentration. G6P was not obtained from this assay, but was determined separately.
Free glucose and G6P can be determined using the above assay on undiluted muscle perchloric acid extract.

‡ relative fluorescence = (sample fluorescence - respective blank fluorescence)
Free glucose, glucose-1-Phosphate (G1P), glucose-6-Phosphate (G6P) and fructose-6-Phosphate (F6P)

Principle:

\[
\begin{align*}
\text{Glucose} + \text{ATP} & \xrightarrow{\text{hexokinase}} \text{glucose-6-P} + \text{ADP} \\
\text{Fructose-6-P} & \xrightarrow{\text{P-gluc isomerase}} \text{glucose-6-P} \\
\text{Glucose-1-P} & \xrightarrow{\text{P-glucomutase}} \text{glucose-6-P} \\
\text{NADP}^+ & \xrightarrow{\text{NADPH} + \text{H}^+} \\
\text{Glucose-6-P} & \xrightarrow{\text{glucose-6-P dehydrogenase}} \text{6-P-gluconolactone}
\end{align*}
\]

Buffer: Tris-HCl 50 mmol·l⁻¹, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)
Cofactor: NADP 5 mmol·l⁻¹
Enzymes*: G6P-DH 7 U·ml⁻¹; HK 28 U·ml⁻¹; Phosphoglucomutase (PGluM) 4 U·ml⁻¹; Phosphoglucoisomerase (PGI) 35 U·ml⁻¹
Reagents: Glucose 1,6-bisphosphate (G1,6BP) 0.25 mmol·l⁻¹; Dithiothreitol (DTT) 50 mmol·l⁻¹; MgCl₂ 100 mmol·l⁻¹; EDTA·Na₂ 100 mmol·l⁻¹; ATP 10 mmol·l⁻¹
Standards: G6P 2 mmol·l⁻¹; Glucose 0.505 mmol·l⁻¹
Diluent: Carbonate buffer 20 mmol·l⁻¹, pH 10.0

Working standards were prepared daily as follows:

\[
\begin{array}{cccccccc}
\text{G6P} & \text{mmol·l}^{-1} & 0 & 10 & 30 & 50 & 100 & 300 \\
\text{double distilled water} & \text{mmol·l}^{-1} & 2000 & 1990 & 1970 & 1850 & 1800 & 1700 \\
\text{G6P concentration} & \text{mmol·l}^{-1} & 0 & 10 & 30 & 50 & 100 & 300 \\
\end{array}
\]

Reaction mixture was prepared immediately prior to analysis for 4 sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards. Since the changes in concentration of these metabolites during sprinting are several fold, separate standard concentrations were used for resting and post-exercise samples:

Resting samples: 0, 10, 30, 50 μmol·l⁻¹ and post-exercise samples 0, 10, 30, 50, 100, 300 μmol·l⁻¹

* enzymes were diluted using Tris-HCl 20 mmol·l⁻¹, pH 8.1 with 0.02% BSA
G6P-DH, glucose-6-P dehydrogenase; HK, hexokinase; PGluM, Phosphoglucomutase; PGI, Phosphoglucoisomerase
APPENDIX B: Muscle metabolite assays

Reaction mixture (final concentrations):

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>1 ml</td>
<td></td>
</tr>
<tr>
<td>NADP</td>
<td>10 µl (48 µmol·l⁻¹)</td>
<td></td>
</tr>
<tr>
<td>MgCl₂</td>
<td>10 µl (0.955 mmol·l⁻¹)</td>
<td></td>
</tr>
<tr>
<td>DTT</td>
<td>10 µl (0.478 mmol·l⁻¹)</td>
<td></td>
</tr>
<tr>
<td>EDTA·Na₂</td>
<td>5 µl (0.478 mmol·l⁻¹)</td>
<td></td>
</tr>
<tr>
<td>G1,6BP</td>
<td>2 µl (0.5 µmol·l⁻¹)</td>
<td></td>
</tr>
<tr>
<td>G6P-DH</td>
<td>10 µl (0.067 U·ml⁻¹)</td>
<td></td>
</tr>
</tbody>
</table>

Procedure:

1. 10 µl aliquots of the undiluted extract were pipetted into 8 fluorimeter tubes resulting in 4 sets of duplicate samples. Four sets of double distilled water blanks and 1 set of standards were also pipetted (10 µl aliquots).

2. 200 µl of the above reaction mixture was added to one set of tubes, blanks and G6P standards (G6P determination; ASSAY 1).

3. The remaining reaction mixture was divided into 3 equal parts and one of the 3 different enzymes was added to each part:
   a) 5 µl of HK (0.131 U·ml⁻¹) and 20 µl of ATP (0.187 mmol·l⁻¹) was added per ml of reaction mixture and 200 µl of this reaction mixture was pipetted to the second set of tubes and blanks (Glucose+G6P determination; ASSAY 2).
   b) 10 µl of PGI (0.038 U·ml⁻¹) was added per ml of reaction mixture and 200 µl of this reaction mixture was pipetted to the third set of tubes and blanks (G1P+G6P determination; ASSAY 3).
   c) 10 µl of PG1 (0.331 U·ml⁻¹) was added per ml of reaction mixture and 200 µl of this reaction mixture was pipetted to the fourth set of tubes and blanks (F6P+G6P determination; ASSAY 4).

4. After incubating for 30-40 min at room temperature, 1 ml of carbonate buffer was added to each tube using a Hamilton automatic dispenser, and after thorough mixing fluorescence was read.

For Glucose, G1P and F6P determination: The relative fluorescence (sample fluorescence - respective blank fluorescence) of G6P (ASSAY 1) was subtracted from the relative fluorescence of each of the other 3 assays. Glucose standards (0-200 µmol·l⁻¹) made from the 0.505 mmol·l⁻¹ glucose standard, were also used occasionally together with the G6P standards.

The concentrations of Glucose, G6P, G1P and F6P were determined using simple linear regression analysis of the standard concentrations (G6P and Glucose) on fluorescence readings.

Concentrations were corrected for dilution during the extraction procedure:

Concentration (mmol·kg dry muscle⁻¹): regression value x 0.125
APPENDIX B: Muscle metabolite assays

**Pyruvate (by development of fluorescence in strong alkali)**

**Principle:**

\[ \text{Pyruvate} \xrightarrow{\text{NADH} + \text{H}^+} \text{NAD}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{lactate} \]

Buffer: Imidazole-HCl 50 mmol·l⁻¹, pH 7.0
Cofactor: NADH (grade I) 5 mmol·l⁻¹
Enzyme: Lactate dehydrogenase (LDH) 5500 U·ml⁻¹ (undiluted)
Reagents: EDTA·Na₂ 100 mmol·l⁻¹; HCl 4 mol·l⁻¹; NaOH 6 mol·l⁻¹
Standards: Pyruvate 2 mmol·l⁻¹

Working standards were prepared daily as follows:

<table>
<thead>
<tr>
<th>Pyruvate concentration (µmol·l⁻¹)</th>
<th>0</th>
<th>10</th>
<th>30</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>double distilled water (µl)</td>
<td>2000</td>
<td>1990</td>
<td>1970</td>
<td>1950</td>
<td>1900</td>
</tr>
</tbody>
</table>

Reaction mixture was prepared immediately prior to analysis for 2 sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

**Reaction mixture (final concentrations):**

Buffer 1 ml
NADH 2 µl (10 µmol·l⁻¹)
EDTA·Na₂ 10 µl (0.988 mmol·l⁻¹)
APPENDIX B: Muscle metabolite assays

Procedure:

1. 10μl aliquots of undiluted extract were pipetted into 2 pairs of fluorimeter tubes resulting in 2 sets of duplicate samples. A set of double distilled water blanks and a set of standards were also pipetted (10 μl aliquots).

2. 200 μl of the above reaction mixture was added to one set of tubes and blanks (extract blanks; STEP 1).

3. 2 μl of LDH (10.85 U·ml⁻¹) was added per ml of remaining reaction mixture and 200 μl of this reaction mixture was then added to the second set of tubes, blanks and standards (extract blanks+pyruvate determination; STEP 2).

Furthermore, 1 ml of this reaction mixture was added to 50 μl of 100 μmol·l⁻¹ pyruvate standard and the reaction was followed in the fluorimeter and recorded on a chart recorder, to ensure that the reaction had reached completion.

4. After incubating for 40 min at room temperature, 20 μl of HCl 4 mol·l⁻¹ was added to each tube using a positive displacement pipette, and each tube was mixed immediately and left at room temperature for 5 min. Addition of acid destroyed the remaining NADH without affecting NAD (Lowry and Passonneau, 1972).

5. Following the 5 min, 1 ml of NaOH 6 mol·l⁻¹ was added in each tube, and the tube was mixed immediately and thoroughly. Tubes were then incubated for 60 min in a dark cupboard. The destruction of NAD in strong alkali (NaOH) results in the formation of highly fluorescent products which are, however, light sensitive [so incubation is in the dark] (Lowry and Passonneau, 1972).

6. Following the 60 min incubation, fluorescence was read. Tubes were exposed to a minimum of incident light to prevent decay in fluorescence.

For pyruvate determination: The relative fluorescence (sample fluorescence - respective blank fluorescence) of extract blanks (STEP 1) was subtracted from the relative fluorescence of STEP 2. The concentration of pyruvate was determined using simple linear regression analysis of the standard concentrations (pyruvate) on fluorescence readings. Concentrations were corrected for the dilution during the extraction procedure:

Concentration (mmol·kg dry muscle⁻¹): regression value x 0.125
**Lactate**

**Principle:**

\[
\text{Lactate} \xrightarrow{\text{lactate dehydrogenase}} \text{Pyruvate} \quad \text{NAD}^+ \quad \text{NADH} + \text{H}^+
\]

**Reagents:**

- **Buffer:** Hydrazine 1.1 mol·l⁻¹, pH 9.0 with 1 mmol·l⁻¹ EDTA·Na₂
- **Cofactor:** NAD 50 mmol·l⁻¹
- **Enzyme:** lactate dehydrogenase (LDH) 5500 U·ml⁻¹ (undiluted)
- **Standard:** L-Lactate 2 mmol·l⁻¹
- **Diluent:** Carbonate buffer 20 mmol·l⁻¹, pH 10.0

Working standards were prepared daily as follows:

<table>
<thead>
<tr>
<th>L-Lactate 2 mmol·l⁻¹ (μl)</th>
<th>0</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>150</th>
<th>400</th>
<th>750</th>
<th>1200</th>
</tr>
</thead>
<tbody>
<tr>
<td>double distilled water (μl)</td>
<td>2000</td>
<td>1950</td>
<td>1925</td>
<td>1900</td>
<td>1850</td>
<td>1600</td>
<td>1250</td>
<td>800</td>
</tr>
<tr>
<td>L⁺ Concentration (μmol·l⁻¹)</td>
<td>0</td>
<td>50</td>
<td>75</td>
<td>100</td>
<td>150</td>
<td>400</td>
<td>750</td>
<td>1200</td>
</tr>
</tbody>
</table>

Reaction mixture was prepared immediately prior to analysis for 1 set of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

**Reaction mixture (final concentration):**

- Buffer 1 ml
- NAD 10 μl (0.491 mmol·l⁻¹)
- LDH 8 μl (43.22 U·ml⁻¹)

**Procedure:**

1. 10 μl aliquots of the undiluted extract were pipetted into a set of duplicate fluorimeter tubes. A set of double distilled water blanks and 1 set of standards were also pipetted (10 μl aliquots).
2. 200 μl of the above reaction mixture was added to each tube (mix well). Furthermore, 1 ml of the reaction mixture was added to 50 μl of 1200 μmol·l⁻¹ lactate standard and the reaction was followed in the fluorimeter and recorded on a chart recorder, until it reached completion.
5. After incubating for 30-40 min at room temperature (reaction completed), 1 ml of carbonate buffer was added to each tube using a Hamilton automatic dispenser, and after thorough mixing fluorescence was read. The concentration of lactate was determined using simple linear regression analysis of the standard concentrations (lactate) on fluorescence readings. Since the changes in concentration of lactate during sprinting are several fold, separate standard concentrations were used for resting and post-exercise samples. 

Resting samples: 0, 50, 75, 100, 150 μmol·l⁻¹ and post-exercise samples: 0, 50, 150, 400, 750, 1200 μmol·l⁻¹

Concentrations were corrected for dilution during the extraction procedure:
Concentration (mmol·kg dry muscle⁻¹): regression value x 0.125
**APPENDIX C**

**SPECTROPHOTOMETRIC MUSCLE METABOLITE ASSAYS FOR VALIDATION OF STANDARDS**

**General points**

All assays were carried out in a 1 ml semi-micro cuvette at 340 nm, reading against air, and were followed to completion on a chart recorder. The concentration of the standard was calculated using the following equation:

\[
[C] = \frac{V_c \times \Delta A}{V_a \times 6.22}
\]

where:

- \([C]\) = the concentration of the standard (mmol·l\(^{-1}\))
- \(V_c\) = the final cuvette volume (ml)
- \(V_a\) = the volume of standard added (ml)
- \(\Delta A\) = change in absorbance = \(|\text{initial absorbance }A_1 - \text{final absorbance }A_2| - |\text{absorbance of enzyme}|\)  
  (vertical lines denote absolute values)
- 6.22 = the mmolar extinction coefficient for NADH/NADPH at 340 nm

\* in a disappearance assay (when absorbance decreases during reaction), the absorbance of the enzyme is: subtracted from the sample absorbance if addition of enzyme decreases absorbance, and is added to the sample absorbance if addition of enzyme increases absorbance.

In a normal assay (when absorbance increases during reaction), the absorbance of the enzyme is: added to the sample absorbance if addition of enzyme decreases absorbance, and is subtracted from the sample absorbance if addition of enzyme increases absorbance.
APPENDIX C: Spectrophotometric muscle assays

Adenosine triphosphate (ATP)

Principle:

\[
\text{ATP} + \text{glucose} \xrightarrow{\text{hexokinase}} \text{ADP} + \text{glucose-6-P}
\]

\[
\text{NADP}^+ \xrightarrow{\text{glucose-6-P dehydrogenase}} \text{NADPH} + \text{H}^+
\]

\[
\text{Glucose -6-P} \xrightarrow{\text{glucose-6-P dehydrogenase}} \text{6-P-gluconolactone}
\]

Buffer: Tris-HCl 50 mmol·l⁻¹, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)

Cofactor: NADP 50 mmol·l⁻¹

Enzymes*: G6P-DH 7 U·ml⁻¹; HK 28 U·ml⁻¹

Reagents: Glucose 100 mmol·l⁻¹; MgCl₂ 100 mmol·l⁻¹; Dithiothreitol (DTT) 50 mmol·l⁻¹

Standards: ATP 2 mmol·l⁻¹

Reaction mixture (final concentrations):

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>1 ml</td>
<td></td>
</tr>
<tr>
<td>NADP</td>
<td>10 µl</td>
<td>(0.459 mmol·l⁻¹)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>50 µl</td>
<td>(4.587 mmol·l⁻¹)</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 µl</td>
<td>(0.917 mmol·l⁻¹)</td>
</tr>
<tr>
<td>G6P-DH</td>
<td>10 µl</td>
<td>(0.064 U·ml⁻¹)</td>
</tr>
<tr>
<td>DTT</td>
<td>10 µl</td>
<td>(0.459 mmol·l⁻¹)</td>
</tr>
</tbody>
</table>

Procedure:

1. 1 ml of the above reaction mixture was added to 20 µl of 2 mmol·l⁻¹ ATP standard. A₁ was then read. 10 µl of HK (0.272 U·ml⁻¹) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (4-6 min). A₂ was then read. Further 10 µl of HK were then added and absorbance was read again (A₃) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme (\(A_{\text{ENZYME}} = |A₂ - A₃|\)). The concentration of standard was calculated using the formula (1) described.

---

* enzymes were diluted using Tris-HCl 20 mmol·l⁻¹, pH 8.1 with 0.02% BSA
G6P-DH, glucose-6-P dehydrogenase; HK, hexokinase
**Adenosine diphosphate (ADP)**

**Principle:**

\[
\text{ADP} + \text{P-pyruvate} \xrightarrow{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate}
\]

\[
\text{Pyruvate} \xrightarrow{\text{lactate dehydrogenase}} \text{lactate}
\]

\[
\text{NADH} + \text{H}^+ \xrightarrow{} \text{NAD}^+
\]

**Buffer:** Imidazole-HCl 50 mmol·l⁻¹, pH 7.0

**Cofactor:** NADH (grade I) 5 mmol·l⁻¹

**Enzymes:** PK 75 U·ml⁻¹; LDH 40 U·ml⁻¹

**Reagents:** Phosphoenol pyruvate (PEP) 30 mmol·l⁻¹; MgCl₂ 100 mmol·l⁻¹; KCl 3 mol·l⁻¹; ethylenediamine tetra-acetic disodium salt (EDTA·Na₂) 100 mmol·l⁻¹

**Standards:** ADP 2 mmol·l⁻¹

**Reaction mixture (final concentrations):**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂</td>
<td>20 µl (1.848 mmol·l⁻¹)</td>
</tr>
<tr>
<td>KCl</td>
<td>25 µl (69.316 mmol·l⁻¹)</td>
</tr>
<tr>
<td>EDTA·Na₂</td>
<td>2 µl (0.185 mmol·l⁻¹)</td>
</tr>
<tr>
<td>NADH</td>
<td>15 µl (0.069 mmol·l⁻¹)</td>
</tr>
<tr>
<td>PK</td>
<td>75 µl (0.293 U·ml⁻¹)</td>
</tr>
<tr>
<td>LDH</td>
<td>40 µl (0.37 U·ml⁻¹)</td>
</tr>
</tbody>
</table>

**Procedure:**

1. 1 ml of the above reaction mixture was added to 20 µl of 2 mmol·l⁻¹ ADP standard. A₁ was then read. 4 µl of PK (0.293 U·ml⁻¹) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (2-4 min). A₂ was then read. Further 4 µl of PK were then added and absorbance was read again (A₃) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme \( A_{\text{ENZYME}} = |A₂ - A₃| \). The concentration of standard was calculated using the formula (1) described.

* enzymes were diluted using Tris-HCl 20 mmol·l⁻¹, pH 8.1 with 0.02% BSA

PK, pyruvate kinase; LDH, lactate dehydrogenase
APPENDIX C: Spectrophotometric muscle assays

**Phosphocreatine (PCr)**

**Principle:**

\[
P\text{-Creatine} + ADP \xrightarrow{\text{creatine kinase}} \text{creatine} + ATP
\]

\[
\text{ADP} + \text{glucose} \xrightarrow{\text{hexokinase}} \text{ADP} + \text{glucose-6-P}
\]

\[
NADP^+ \xrightarrow{\text{6-P-gluconolactone dehydrogenase}} \text{NADPH} + H^+
\]

\[
\text{Glucose-6-P} \xrightarrow{\text{6-P-gluconolactone dehydrogenase}} \text{6-P-gluconolactone}
\]

**Buffer:** Tris-HCl 50 mmol·l⁻¹, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)

**Cofactor:** NADP 50 mmol·l⁻¹

**Enzymes:** G6P-DH 7 U·ml⁻¹; HK 28 U·ml⁻¹; CK 1290 U·ml⁻¹

**Reagents:** Glucose 100 mmol·l⁻¹; MgCl₂ 100 mmol·l⁻¹; Dithiothreitol (DTT) 50 mmol·l⁻¹; ADP 50 mmol·l⁻¹

**Standards:** PCr 2 mmol·l⁻¹

**Reaction mixture (final concentrations):**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>1 ml</td>
<td></td>
</tr>
<tr>
<td>NADP</td>
<td>10 μl</td>
<td>(0.450 mmol·l⁻¹)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>50 μl</td>
<td>(4.505 mmol·l⁻¹)</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 μl</td>
<td>(0.901 mmol·l⁻¹)</td>
</tr>
<tr>
<td>ADP</td>
<td>10 μl</td>
<td>(0.450 mmol·l⁻¹)</td>
</tr>
<tr>
<td>DTT</td>
<td>10 μl</td>
<td>(0.450 mmol·l⁻¹)</td>
</tr>
<tr>
<td>G6P-DH</td>
<td>10 μl</td>
<td>(0.063 U·ml⁻¹)</td>
</tr>
<tr>
<td>HK</td>
<td>10 μl</td>
<td>(0.252 U·ml⁻¹)</td>
</tr>
</tbody>
</table>

**Procedure:**

1. 1 ml of the above reaction mixture was added to 20 μl of 2 mmol·l⁻¹ PCr standard. A₁ was then read. 10 μl of CK (12.524 U·ml⁻¹) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (10-12 min). A₂ was then read. Further 10 μl of CK were then added and absorbance was read again (A₃) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme (\( A_{\text{ENZYME}} = |A₂ - A₃| \)). The concentration of standard was calculated using the formula (1) described.

---

*enzymes were diluted using Tris-HCl 20 mmol·l⁻¹, pH 8.1 with 0.02% BSA
G6P-DH, glucose-6-P dehydrogenase; HK, hexokinase; CK, creatine kinase*
Creatine

Principle:

\[
\begin{align*}
\text{Creatine} + \text{ATP} & \xrightarrow{\text{creatine kinase}} \text{P-Creatine} + \text{ADP} \\
\text{ADP} + \text{P-pyruvate} & \xrightarrow{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate} \\
\text{NADH} + \text{H}^+ & \xrightarrow{\text{NAD}^+} \text{ATP} + \text{pyruvate} \\
\text{Pyruvate} & \xrightarrow{\text{lactate dehydrogenase}} \text{lactate}
\end{align*}
\]

Buffer: Imidazole-HCl 50 mmol·l⁻¹, pH 7.5
Cofactor: NADH (grade I) 5 mmol·l⁻¹
Enzymes*: CK 1260 U·ml⁻¹; PK 75 U·ml⁻¹; LDH 50 U·ml⁻¹
Reagents: ATP 100 mmol·l⁻¹; phosphoenol pyruvate (PEP) 30 mmol·l⁻¹; MgCl₂ 100 mmol·l⁻¹; KCl 3 mol·l⁻¹;
Standards: Creatine 2 mmol·l⁻¹

**Reaction mixture (final concentrations):**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂</td>
<td>50 μl (4.523 mmol·l⁻¹)</td>
</tr>
<tr>
<td>KCl</td>
<td>10 μl (27.137 mmol·l⁻¹)</td>
</tr>
<tr>
<td>ATP</td>
<td>10 μl (0.905 mmol·l⁻¹)</td>
</tr>
<tr>
<td>NADH</td>
<td>15 μl (0.068 mmol·l⁻¹)</td>
</tr>
<tr>
<td>PEP</td>
<td>6.5 μl (0.176 mmol·l⁻¹)</td>
</tr>
<tr>
<td>LDH</td>
<td>4 μl (0.181 U·ml⁻¹)</td>
</tr>
<tr>
<td>PK</td>
<td>10 μl (0.678 U·ml⁻¹)</td>
</tr>
</tbody>
</table>

**Procedure:**

1. 1 ml of the above reaction mixture was added to 20 μl of 2 mmol·l⁻¹ Creatine standard. A₁ was then read. 10 μl of CK (12.233 U·ml⁻¹) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (10-30 min). A₂ was then read. Further 10 μl of CK were then added and absorbance was read again (A₃) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme \( \Delta \text{ENZYME} = \left| A_2 - A_3 \right| \). The concentration of standard was calculated using the formula (1) described.

*enzymes were diluted using Tris-HCl 20 mmol·l⁻¹, pH 8.1 with 0.02% BSA
CK, creatine kinase; PK, pyruvate kinase; LDH, lactate dehydrogenase
Glucose-6-Phosphate (G6P)

Principle:

\[
\text{Glucose-6-P} \xrightarrow{\text{glucose-6-P dehydrogenase}} \text{NADPH} + \text{H}^+ \xrightarrow{\text{NADP}^+} \text{6-P-gluconolactone}
\]

Buffer: Tris-HCl 100 mmol·I⁻¹, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)
Cofactor: NADP 5 mmol·I⁻¹
Enzymes: Glucose-6-P dehydrogenase (G6P-DH) 7 U·ml⁻¹
          (diluted using Tris-HCl 20 mmol·I⁻¹, pH 8.1 with 0.02% BSA)
Reagents: EDTA·Na₂ 100 mmol·l⁻¹
Standard: G6P 2 mmol·l⁻¹

Reaction mixture (final concentrations):
Buffer 1 ml
NADP 10 µl (0.493 mmol·l⁻¹)  EDTA·Na₂ 5 µl  (0.493 mmol·l⁻¹)

Procedure:
1. 1 ml of the above reaction mixture was added to 20 µl of 2 mmol·l⁻¹ G6P standard. A₁ was then read. 10 µl of G6P-DH (0.068 U·ml⁻¹) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (3-5 min). A₂ was then read. Further 10 µl of G6P-DH were then added and absorbance was read again (A₃) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme (\(A_{\text{ENZYM}} = |A_2 - A_3|\)). The concentration of standard was calculated using the formula (1) described.
APPENDIX C: Spectrophotometric muscle assays

Lactate

Principle:  

\[ \text{NAD}^+ \xrightarrow{\text{Lactate dehydrogenase}} \text{NADH} + \text{H}^+ \]

\[ \text{Lactate} \xrightarrow{\text{dehydrogenase}} \text{Pyruvate} \]

Reagents:
Buffer: Hydrazine 1.1 mol·l⁻¹, pH 9.0 with 1 mmol·l⁻¹ EDTA·Na₂
Cofactor: NAD 50 mmol·l⁻¹
Enzyme: lactate dehydrogenase (LDH) 5500 U·ml⁻¹ (undiluted)
Standard: L-Lactate 2 mmol·l⁻¹

Reaction mixture (final concentration):
Buffer 1 ml
NAD 40 µl (1.923 mmol·l⁻¹)

Procedure:
1. 1 ml of the above reaction mixture was added to 20 µl of 2 mmol·l⁻¹ lactate standard. \( A_1 \) was then read. 5 µl of LDH (27.363 U·ml⁻¹) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (20-30 min). \( A_2 \) was then read. Further 5 µl of LDH were then added and absorbance was read again \( (A_3) \) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme \( (A_{\text{ENZYME}} = |A_2 - A_3|) \). The concentration of standard was calculated using the formula (1) described.
BUFFERS AND REAGENTS FOR MUSCLE METABOLITE ASSAYS

This appendix contains some examples on how to make buffers and solutions for muscle metabolite assays.

**Perchloric acid (HClO₄)** 0.5 M with 1 mM EDTA·Na₂ (for muscle metabolites extraction procedure)

**NECESSARY INFORMATION**

- % max assay HClO₄ (on the bottle), eg. HClO₄ 60%, 70%, etc. (w/w). This means that there are 60 g of HClO₄ per 100 g of solution.
- density of solution (on the bottle), eg. 1.54 g·ml⁻¹. This means that every ml of the solution has a mass of 1.54 g.
- molecular weight of chemical (on the bottle), eg. HClO₄ = 100.46

**TO FIND MOLARITY OF SOLUTION**

- eg. for 60% HClO₄; density 1.54 g·ml⁻¹
  \[ \text{1 litre of solution has a mass of 1540 g, and contains } 1540 \times 0.60 \text{ or 924 g of HClO}_4. \]
- In order to find the molarity of the solution, divide the above number by the molecular weight of HClO₄, i.e. \( \frac{924}{100.46} = 9.198 \text{ molar (M)} \).

**PREPARATION OF SOLUTION**

- For 250ml of 0.5 M HClO₄ from HClO₄ 60% (9.198 M):
  - divide 9.198 by 0.5 to find dilution factor = 18.396 - fold dilution.
  - divide desired volume of solution (e.g. 250 g) by the dilution factor, i.e. \( \frac{250}{18.396} = 13.59 \text{ ml} \).
  \[ \therefore 13.59 \text{ ml of HClO}_4 \text{ 60% (9.197 M) must be put in a 250 ml flask, and then the flask must be filled with distilled water up to the 250 ml mark.} \]
- For the extraction procedure of muscle metabolites, this solution must also contain 1 mmol·l⁻¹ EDTA·Na₂.
- EDTA·Na₂ has a molecular weight of 372.24. This means that a one molar (M) solution contains 372.24 g EDTA·Na₂ in 1 litre of solution.
- For a 1mM concentration of EDTA·Na₂, 0.37224 g must be dissolved in 1 litre of solution. The volume of our solution is 250 ml, so we need to add 1/4 of 0.37224 g = 0.09306 g EDTA·Na₂.

**Tris-HCl buffer 50 mM, pH 8.1 with 0.02% Bovine Serum Albumin**

**GENERAL**

A buffer solution is one that resists a change in pH on the addition of acid or alkali. Most commonly, the buffer solution consists of a mixture of a weak Brönsted acid and its conjugate base. Two factors determine the effectiveness of a buffer solution: (a) the molar concentration of the buffer components (sum of the concentration of the weak acid and its conjugate base) and (b) its dissociation constant (that is the equilibrium constant of the dissociation reaction, usually given as its negative logarithm (pK)). In practice, useful buffering is limited to one pH unit either side of the pK value (Lowry and Passonneau, 1972; Dawson et al., 1969). Therefore, selection of a buffer system must be such that the pK of the buffer is very close to the desired pH (Conn and Stumpf, 1972). The Henderson-Hasselbalch equation relates pH and pK† with the ratio of the conjugate base to the weak acid:

\[
pH = pK + \log \frac{\text{concentration of conjugate base (salt)}}{\text{concentration of undissociated acid}}
\]

† It must be noted that pK values used for buffer pH calculations have been determined at certain temperatures (usually 25 °C) and for “infinite dilution” (zero ionic strength). Therefore, observed pK’s are always lower than these “true” pK’s (Bates, 1964; Lowry and Passonneau, 1972), and the pH of the buffer usually has to be fine-adjusted by addition of acid or alkali. It is best to adjust the pH of the concentrated buffer so that when it is diluted 10-50 fold in an assay, the pH of the final reaction mixture is known precisely (Conn and Stumpf, 1972).
NECESSARY INFORMATION (Tris-HCl buffer 50 mM, pH 8.1 with 0.02% BSA)

Buffer made of the primary amine tris - (hydroxymethyl)amino methane or “Tris” (NH2C(CH2OH)3 and Hydrochloric acid.
The pKₐ of Tris is 8.0 and the desired pH is 8.1.

\[
pH = pK_a + \log \frac{[\text{base}]}{[\text{acid}]} \quad \rightarrow \quad 8.1 - 8.0 = \log \frac{[\text{free amine}]}{[\text{acid salt}]} \quad \rightarrow
\]

\[
10^{0.1} = \frac{[\text{free amine}]}{[\text{acid salt}]} \quad \rightarrow \quad 1.26 = \frac{[\text{free amine}]}{[\text{acid salt}]}
\]

Therefore, the ratio of base : acid must be 1.26 : 1, or in percentages = 2.26 (total volume of solution) is 100%

\[
x = \frac{(100 \times 1.26)}{2.26} = 55.75 \% \text{ of total buffer will be as free amine and } 44.25 \% \text{ as the acid salt}
\]

.: 1 litre of 50 mM Tris-HCl will contain 50 mM of free amine (Tris) and 44.25% of 50 mM or 22.125 mM of acid salt.
Molecular weight of Tris: = 121.14

.: For a concentration of 50mM (121.14 x 50)/1000 = 6.057 g of solid Tris in 1 litre.
The molarity of the HCl solution has been determined below as 10.18 M.

.: 22.125 mM or 2.173 ml per litre of 10.18 M HCl have to be added.
We also need to add 0.02% Bovine Serum Albumin (BSA) in the solution.

.: BSA : 0.2 g per 1000 ml of solution.
In summary, 0.022125 moles HCl were added in 0.05 moles Tris per liter of distilled water + 0.2 g BSA.

Find molarity of Hydrochloric acid (HCl)

Molecular weight: 36.46; % assay: 32.0 % HCL; Specific gravity: 1.16 g·ml⁻¹.

MOLARITY OF HCL

1 litre of solution has a mass of 1160 g, and contains 1160 x 0.32 or 371.2 g of HCl. To find the molarity of HCl, divide the above number by the molecular weight of HCl, i.e. 371.2/36.46 = 10.18 molar (M).
Hydrazine buffer 1.1 M, pH 9.0 with 1 mM EDTA-Na₂ (for muscle and blood lactate)

Buffer made of Hydrazine hydrate (base) and Hydrazinium sulphate (acid)

\[ \text{NH}_2\text{NH}_2\text{H}_2\text{O} \text{ (Hydrazine Hydrate)} \]
\[ \text{NH}_2\text{NH}_2\text{H}_2\text{SO}_4 \text{ (Hydrazinium Sulphate)} \]

The pKa of Hydrazine is 8.23 (Lowry and Passonneau, 1972), therefore a 50 : 50 concentration of Hydr. Hydrate and Hydrazinium Sulphate would give a pH of 8.23.

Using the Henderson - Hasselbalch equation, we can calculate the excess base required to increase the pH of the solution from 8.23 to 9.0.

\[ \text{pH} = \text{pK}_a + \log \frac{[\text{base}]}{[\text{acid}]} \]

\[ 9.0 - 8.23 = \log \frac{[\text{base}]}{[\text{acid}]} \]

\[ 10^{0.77} = \frac{[\text{base}]}{[\text{acid}]} \]

Therefore, the ratio of base : acid must be 5.89 : 1, or in percentages = 6.89 total volume of solution, is 100%

\[ x = \frac{(100 \times 5.89)}{6.89} = 85.49 \% \text{ of total buffer concentration will be Hydrazine hydrate and 14.51} \% \text{ Hydrazinium sulphate} \]

Molarity of Hydrazine Hydrate

Hydrazine Hydrate is in solution 99% max assay and 1.03 g·ml⁻¹.

\[ \therefore 1 \text{ litre of solution has a mass of 1030 g, and contains } 1030 \times 0.99 = 1019.7 \text{ g of Hydrazine Hydrate.} \]

To find the molarity of Hydrazine Hydrate, divide the above number by the molecular weight of Hydrazine Hydrate, i.e. 1019.7/50.06 = 20.37 molar (M).

Preparation of Buffer

Hydrazine hydrate concentration (base) must be 85.49 % of the total buffer concentration (1.1 M) or 0.940 M (46.167 ml per litre of buffer)

Hydrazinium sulphate concentration (acid) will be the remaining 14.51 % or 0.160 M (20.77 g per litre of buffer; molecular weight of Hydrazinium sulphate: 130.12).

1mM EDTA·Na₂ (0.37224 g per litre of buffer) is also added in the solution.
**Imidazole buffer 50 mM, pH 7.5 and 7.0 (used in the ADP-AMP, Creatine and Pyruvate assays)**

Buffer made of Imidazole (C₃H₄N₂; molecular weight: 68.08) and Hydrochloric acid. The pKₐ of Imidazole is 7.07 (Lowry and Passonneau, 1972) and the desired pH is either 7.0 or 7.5.

Using the Henderson - Hasselbalch equation, we can calculate the ratio of base to acid forms required to change the pH of the solution as required:

(1) For pH: 7.5

\[
\text{pH} = \text{pK} + \log \frac{[\text{base}]}{[\text{acid}]} \Rightarrow 7.5 - 7.07 = \log \frac{[\text{base}]}{[\text{acid}]} \Rightarrow 0.43 = \log \frac{[\text{base}]}{[\text{acid}]} \Rightarrow \frac{[\text{base}]}{[\text{acid}]} = 2.692
\]

Therefore, the ratio of base : acid must be 2.692 : 1, or in percentages =

\[
\frac{(100 \times 2.692)}{3.692} = 72.91 \% \text{ of total Imidazole concentration in the buffer will be present as base and 27.09 \% as the acid form.}
\]

∴ 1 litre of 50 mM Imidazole, pH 7.5 will contain 50 mM of Imidazole (3.405 g per litre of buffer) and 27.09 % of 50 mM or 13.545 mM of Imidazole in the acid form (i.e. 1.331 ml HCl 10.18 M per litre of buffer).

(2) For pH: 7.0

\[
\text{pH} = \text{pK} + \log \frac{[\text{base}]}{[\text{acid}]} \Rightarrow 7.0 - 7.07 = \log \frac{[\text{base}]}{[\text{acid}]} \Rightarrow -0.07 = \log \frac{[\text{base}]}{[\text{acid}]} \Rightarrow \frac{[\text{base}]}{[\text{acid}]} = 1.18
\]

Therefore, the ratio of acid : base must be 1.18 : 1, or in percentages =

\[
\frac{(100 \times 1.18)}{2.18} = 54.0 \% \text{ of total Imidazole concentration in the buffer will be present in the acid form and 46.0 \% will be present in the base form.}
\]
APPENDIX D: Buffers and reagents for muscle metabolite assays

1 litre of 50 mM Imidazole, pH 7.0 will contain 50 mM of Imidazole (3.405 g per litre of buffer) and 54.0 % of 50 mM or 27 mM of Imidazole in the acid form (i.e. 2.652 ml HCl 10.18 M per litre of buffer).

CARBONATE BUFFER 20mM, pH 10.0 (used as diluent in fluorometric assays)

Buffer made of Sodium bicarbonate (acid) NaHCO₃ and Sodium carbonate (base) Na₂CO₃.

The pKₐ₂ of Carbonic acid (H₂CO₃) is 10.32 (Dawson et al., 1969). Using the Henderson - Hasselbalch equation we can determine the excess acid to lower the pH from 10.33 to 10.0:

\[
pH = \text{PKa} + \log \frac{[\text{base}]}{[\text{acid}]} \rightarrow 10.0 = 10.32 + \log \frac{[\text{base}]}{[\text{acid}]} \rightarrow 10.0-10.32 = \log \frac{[\text{base}]}{[\text{acid}]} \rightarrow -0.32 = \log \frac{[\text{base}]}{[\text{acid}]} \rightarrow 0.32 = \log \frac{[\text{acid}]}{[\text{base}]} \rightarrow 10^{0.32} = \frac{[\text{acid}]}{[\text{base}]} \rightarrow 2.09 = \frac{[\text{acid}]}{[\text{base}]}
\]

Therefore, the ratio of acid to base concentration is 2.09 (i.e., in order to have a pH of 10.0, the acid must be 2.09 times more than the base. In percentages:

If \(3.09 = \text{total volume of solution (100%)}\)

\[2.09 \text{ (acid)} \times x = \frac{(100 \times 2.09)}{3.09} = 67.64 \% \text{ of total buffer concentration will be Sodium bicarbonate and 32.36 \% Sodium Carbonate}
\]

Molecular weights: Sodium bicarbonate (84.01) Sodium Carbonate (105.99)

We, therefore, need:

67.64% of 20 mM or 13.528 mM (1.136 g NaHCO₃ per litre of buffer), and
32.36% of 20 mM or 6.472 mM (0.686 g Na₂CO₃ per litre of buffer)
APPENDIX E

CALCULATIONS, CONVERSIONS AND FORMULAE

Hydrogen ion concentration ([H⁺]) - pH conversions

The difference between concentration and activity must be recognised. The concentration of a substance does not always accurately reflect its reactivity in a chemical reaction, particularly if the substance is an electrolyte (Bates, 1964). These discrepancies in behaviour are appreciable when the concentration of the substance is high. Under these conditions the individual particles of the substance may exert a mutual attraction on each other or exhibit interactions with the solvent in which the reaction occurs (Conn and Stumpf, 1972). On the other hand in dilute solution or low concentration, the interactions are considerably less if not negligible. In order to correct for the difference between concentration (C) and activity (a), the activity coefficient (γ) was introduced:

\[ a = C \times \gamma \]

In very dilute concentrations the activity coefficient approaches unity because there is little, if any, solute-solute interaction. At infinite dilution the activity and the concentration are the same. The H⁺ concentration in muscle tissue and blood (even in extreme situations) is approximately between 10⁻⁶ and 10⁻⁷ mol·l⁻¹, at which concentrations the activity coefficient would be unity. In 1909, Sørensen introduced the term pH which expresses the activity of H⁺ ion (a₁H⁺) by means of a logarithmic function:

\[ \text{pH} = \log \frac{1}{a_{H^+}} = -\log a_{H^+} \]

Since we consider activity and concentration to be the same, pH is the negative logarithm of H⁺ concentration (mol·l⁻¹):

\[ \text{pH} = \log \frac{1}{[H^+]} = -\log [H^+] \text{ or } [H^+] = 10^{-\text{pH}} \]

Therefore, pH 7.0 is translated to a H⁺ concentration of 10⁻⁷ mol·l⁻¹ or 100 nmol·l⁻¹.

To convert H⁺ concentration (nmol·l⁻¹) to pH, express it first in mol·l⁻¹ (divide by 10⁹) and take the reciprocal \( \left( \frac{1}{[H^+] \times 10^9} \right) \), and then take its logarithm.
Calculation of the mono and di-protonated forms of Inorganic Phosphate (Pi)

Phosphoric acid (H₃PO₄) is a polyprotic acid, which means that it can yield 3 protons on complete ionization of a mole (Conn and Stumpf, 1972). Therefore H₃PO₄ has 3 pK's (= 2, 7 and 12; Dawson et al., 1969), one for each proton:

\[
\begin{align*}
H₃PO₄ & \leftrightarrow H^+ + H₂PO₄^- & pK₁ = 2 \\
H₂PO₄^- & \leftrightarrow H^+ + HPO₄^{2-} & pK₂ = 6.8^* \\
HPO₄^{2-} & \leftrightarrow H^+ + PO₄^{3-} & pK₃ = 12
\end{align*}
\]

\(^*\) at 38°C (Lawson and Veech, 1979)

At pH between 7.0 and 6.2 (muscle cell) phosphate exists primarily in the \(H₂PO₄^-\) and \(HPO₄^{2-}\) forms, with more diprotonated \((H₂PO₄^-)\) form at the lower pH. The percentage of inorganic phosphate (Pi) in the diprotonated form can be calculated using the Henderson-Hasselbalch equation, the \(pK₂ = 6.8\), and the pH (e.g. 7.0):

\[
\text{pH} = pK_a + \log \frac{[HPO₄^{2-}]}{[H₂PO₄^-]} \rightarrow 7.0 - 6.8 = \log \frac{[HPO₄^{2-}]}{[H₂PO₄^-]} \rightarrow 10^{0.2} = \frac{[HPO₄^{2-}]}{[H₂PO₄^-]} \rightarrow
\]

\[
\%[H₂PO₄^-] = \frac{100}{[1 + 10^{0.2}]}
\]

The absolute value of the diprotonated form can then be calculated if we know the total inorganic phosphate (Pi).

Calculation of hydrogen ion absorption by phosphocreatine (PCr) breakdown

About 1/3 of the total buffering capacity of human muscle \textit{in vivo} is due to \(H^+\) consumption from the creatine kinase reaction. The hydrolysis of ATP results in a decrease in the ATP/ADP ratio, which in turn results in PCr breakdown. The two reactions can be summed as follows:

\[
\begin{align*}
\text{ATP}^{4-} + \text{H}_2\text{O} & \rightarrow \text{ADP}^{3-} + \text{Pi}^{2-} + n\text{H}^+ \\
\text{PCr}^{2-} + \text{ADP}^{3-} + a\text{H}^+ & \rightarrow \text{ATP}^{4-} + \text{Creatine}
\end{align*}
\]

\(pK_{PCr} = 4.5 \quad pK_{Pi} = 6.8\)
The stoichiometric coefficient \( \alpha \) for H\(^+\) release during ATP hydrolysis depends upon the ionic state of ATP, ADP, and Pi and thus upon the concentration of Mg\(^{2+}\), K\(^+\) and H\(^+\) complex-bound to the adenine nucleotides. For an uncomplexed concentration of Mg\(^{2+}\) and K\(^+\) of 1 mM and 160 mM, respectively, the amount of H\(^+\) released per mole ATP hydrolysed will be 0.52 and 0.03 at pH 7.0 and 6.4 (Hultman and Sahlin, 1980). The stoichiometric coefficient \( \alpha \) for H\(^+\) uptake during PCr breakdown is a function of pH and can be calculated using the Henderson-Hasselbalch equation and the pK's of PCr (4.5) and Pi (6.8). The H\(^+\) uptake is due to the differences in pK between PCr and Pi, and demonstrates the buffering capacity of Pi:

At pH = 7.0

\[
\text{PCr}^{2-} + \text{H}_2\text{O} + \alpha \text{H}^+ \rightarrow \text{Creatine} + \text{Pi}^{2-}
\]

\[ pK_{\text{PCr}} = 4.5 \quad pK_{\text{Pi}} = 6.8 \]

\[
\frac{1}{[1 + 10^{pH-pK_{\text{PCr}}}]} \rightarrow \frac{1}{[1 + 10^{pH-pK_{\text{Pi}}}]} 
\]

\[
\frac{1}{[1 + 10^{7.0-4.5}]} \rightarrow \frac{1}{[1 + 10^{7.0-6.8}]} = 0.003 \text{ mol} \rightarrow 0.387 \text{ mol}
\]

\[ \therefore \text{The increase in the protonated forms } (0.387 - 0.003 = 0.384 \text{ mol}), \]

represents the amount of protons taken up (buffered) per mole of PCr broken down, at pH = 7. At pH = 6.4, this increases to 0.703. Thus the value of the stoichiometric coefficient \( \alpha \) is between 0.38 and 0.70 at pH 7.0 and 6.4.
**Conversions of reference bases for expression of muscle metabolite concentrations**

Muscle water content is about 77% w/w (Bangsbo et al., 1992) and therefore when muscle is dried its weight will be 23% of the initial "wet weight". Therefore, all the metabolites contained in 1 kg of wet muscle, will now be contained in 1 x 0.23 = 0.23 kg of dried muscle.

- To convert from [wet] to [dry] → divide concentration in wet by 0.23 (or multiply by 1/0.23 = 4.35).
  
e.g. [ATP]_{WET} : 6.21 mmol·kg⁻¹ → 27 mmol·kg⁻¹

- To convert from [dry] to [wet] → multiply concentration in dry by 0.23 (or divide by 1/0.23 = 4.35).
  
e.g. [ATP]_{DRY} : 27 mmol·kg⁻¹ → 6.21 mmol·kg⁻¹

A muscle water content of 77% w/w means that every kg of dry muscle corresponds to 4.35 kg of wet muscle, or to 3.35 kg of muscle water. Therefore, all the metabolites contained in 1 kg of dry muscle, will also be contained in 4.35 kg of wet muscle, or to 3.35 kg of muscle water.

- To convert from [dry] to [muscle water] → multiply concentration in dry by 0.23/0.77 = 0.3 (or divide by 3.35).
  
e.g. [Pi]_{DRY} : 60 mmol·kg⁻¹ → 17.92 mmol·l⁻¹

- To convert from [wet] to [muscle water] → divide concentration in wet by 0.77 (or multiply by 4.35/3.35 = 1.3).
  
e.g. [Pi]_{WET} : 13.8 mmol·kg⁻¹ → 17.92 mmol·l⁻¹

- To convert from [muscle water] to [dry] → divide concentration in muscle water by 0.23/0.77 = 0.3 (or multiply by 3.35).
  
e.g. [Pi]_{water} : 17.92 mmol·l⁻¹ → 60 mmol·kg⁻¹

- To convert from [muscle water] to [wet] → multiply concentration in muscle water by 0.77 (or divide by 4.35/3.35 = 1.3).
  
e.g. [Pi]_{water} : 17.92 mmol·l⁻¹ → 13.8 mmol·kg⁻¹

However in some cases, the concentration of metabolites in the intracellular water needs to be calculated. Methods like magnetic resonance spectroscopy (³¹P-MRS) measure intracellular metabolites directly. To calculate intracellular concentrations from muscle biopsy data,
the amount of intracellular water must be known. Sjogaard and Saltin (1982) have used the tracer $[^3]H$inulin, and estimated the extracellular water of the vastus lateralis muscle in humans. It was found that: (i) intracellular water (total water - extracellular water) represented $\approx 90\%$ of total muscle water, and (ii) the volume of intracellular water was not significantly increased ($\approx 7\%$, n.s.) after intense supramaximal cycling, even though total muscle water increased by $\approx 15\%$ (Sjogaard and Saltin, 1982).

Therefore, 1 kg of dry muscle corresponds to $\approx 3$ litres of intracellular water ($3.35 \times 90\%$). Expressed as a percentage of the wet muscle weight, intracellular water represents $= 69\%$ of wet weight ($77 \times 90\%$).

- To convert from [dry] to [intracellular water] $\rightarrow$ multiply concentration in dry by $0.23/0.69 = 0.33$ (or divide by 3.0).
  
  e.g. $[Pi]_{DRIY}: 60 \text{ mmol} \cdot \text{kg}^{-1} \rightarrow 20 \text{ mmol} \cdot \text{l}^{-1}$

- To convert from [wet] to [intracellular water] $\rightarrow$ divide concentration in wet by 0.69 (or multiply by $4.35/3.0 = 1.45$).
  
  e.g. $[Pi]_{WET}: 13.8 \text{ mmol} \cdot \text{kg}^{-1} \rightarrow 20 \text{ mmol} \cdot \text{l}^{-1}$

- To convert from [intracellular water] to [dry] $\rightarrow$ divide concentration in intracellular water by $0.23/0.69 = 0.33$ (or multiply by 3.0).
  
  e.g. $[Pi]_{intracell}: 20 \text{ mmol} \cdot \text{l}^{-1} \rightarrow 60 \text{ mmol} \cdot \text{kg}^{-1}$

- To convert from [intracellular water] to [wet] $\rightarrow$ multiply concentration in intracellular water by 0.69 (or divide by $4.35/3.0 = 1.45$).
  
  e.g. $[Pi]_{intracell}: 20 \text{ mmol} \cdot \text{l}^{-1} \rightarrow 13.8 \text{ mmol} \cdot \text{kg}^{-1}$

- To convert from [intracellular water] to [muscle water] $\rightarrow$ multiply concentration in intracellular water by 0.90 (or divide by $3.35/3.0 = 1.11$).
  
  e.g. $[Pi]_{intracell}: 20 \text{ mmol} \cdot \text{l}^{-1} \rightarrow 18 \text{ mmol} \cdot \text{l}^{-1}$

- To convert from [muscle water] to [intracellular water] $\rightarrow$ divide concentration in muscle water by 0.90 (or multiply by $3.35/3.0 = 1.11$).
  
  e.g. $[Pi]_{water}: 18 \text{ mmol} \cdot \text{l}^{-1} \rightarrow 20 \text{ mmol} \cdot \text{l}^{-1}$
Calculation of relative centrifugal force (g) during sample centrifugation

To calculate the relative centrifugal force in multiples of acceleration units (g) at any point along the tube being centrifuged, we need to know the radius (cm) from the centre of the centrifuge spindle to the particular point. In this thesis, that point is taken as the centre of mass of the material being centrifuged. The formula is derived as follows:

\[ F_C = m \times \alpha \rightarrow F_C = m \times \frac{u^2}{r} \rightarrow F_C = m \times \frac{(\omega \times r)^2}{r} \rightarrow F_C = m \times \omega^2 \times r \rightarrow \text{Divide by } m \times g, \text{ to express force relative to the sample's weight: } F_{C_{rel}} = \frac{m \times \omega^2 \times r}{m \times g} \]

\[ F_{C_{rel}} = \frac{\omega^2 \times r}{g} \]

where: \( F_C \), centrifugal force (N); \( m \), mass (kg); \( \alpha \), linear acceleration (m·s\(^{-2}\)); \( u \), linear velocity (m·s\(^{-1}\)); \( r \), radius (m); \( \omega \), angular velocity (rad·s\(^{-1}\)); \( g \), gravitational acceleration (m·s\(^{-2}\)); \( F_{C_{rel}} \), centrifugal force relative to sample's weight (g).

The above formula can be transformed to use more convenient units, e.g. cm for radius and revs·min\(^{-1}\) for angular velocity:

\[ F_{C_{rel}} (g) = 1118 \times 10^{-8} \times R \times V^2 \]

where: \( R \), radius (cm); \( V \), speed of centrifuge (revs·min\(^{-1}\))

Example: \( R = 6 \text{ cm}; V = 13,000 \text{ revs·min}^{-1} \)

\[ F_{C_{rel}} (g) = 11,337 \text{ g} \]
APPENDIX F

MODELLING PHOSPHOCREATINE RESYNTHESIS

Phosphocreatine data are from Chapter IV

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The simple exponential model to describe a subject's phosphocreatine recovery at time \( t \), \( \text{PCr}(t) \), is given by,

\[
\text{PCr}(t) = R - R \cdot \exp(a - b \cdot t),
\]

where \( R \) is the subject's phosphocreatine value at rest, the parameter 'a' indicates the subject's proportion of depleted phosphocreatine at time \( t=0 \) (proportional to their resting value) and the parameter 'b' describes the subject's 'rate of resynthesis'. By rearranging equation (2), a phosphocreatine depletion ratio (PDR) can be defined as,

\[
\text{PDR} = \frac{R - \text{PCr}(t)}{R} = \exp(a - b \cdot t).
\]

Assuming that the exponential model (2) is appropriate to describe phosphocreatine resynthesis, when the natural logarithm of the ratio, \( \ln(\text{PDR}) \), is plotted against time \( t \), the result should be approximately linear. As such, parameters 'a' and 'b' can be estimated using simple linear least-squares regression.

However, supporting the findings of Harris et al. (1976), when the log-transformed phosphocreatine depletion ratio, \( \ln(\text{PDR}) \), was plotted against time \( t \) for the results reported here, the simple exponential model was found to be unsatisfactory for all but one of the subjects (see Fig. F1). Rather than indicating a possible linear model, the subjects' plots would appear to better describe a power function curve, \( \ln(\text{PDR}) = -a \cdot t^b \), where the parameter 'b' would need to be less than unity to accommodate the concave nature of the curves.

The problem with incorporating this power function into either equations (2) or (3), is that the resulting model automatically assumes the subject's phosphocreatine store is entirely depleted at time \( t=0 \), i.e. the subject's phosphocreatine depletion ratio is unity. Although this assumption would appear to be reasonable (Sahlin et al., 1979), an alternative model should allow for an additional parameter, \( a_0 \), that can be fitted and...
subsequently tested for its contribution (and significance) to the prediction of the ratio PDR. Hence, the following model was proposed to represent the phosphocreatine depletion ratio PDR,

\[ PDR = \frac{R - PCr(t)}{R_{\text{initial}}} \exp(-a_0 - at^b) \]  

Unfortunately, after taking natural logarithms, the parameters in equation (4) can no longer be fitted using the usual linear least-squares regression. However, non-linear least-squares routines are now readily available to fit the parameters in such models. When the model (4) was fitted separately to all eight subjects using non-linear least-squares, not surprisingly the quality of fit as measured by the coefficient of determination \( r^2 \) and the standard error, improved substantially in all but one of the subjects' models. Six of the subjects' coefficients of determination \( r^2 \) were greater than 99% leaving the remaining two subjects' \( r^2 \) at 98% and 96%. Of equal importance to the quality of fit, none of the subjects' fitted \( a_0 \) parameters deviated significantly from zero. This finding supports the assumption that PCr is entirely depleted during maximum exercise of this type. Hence, the model for the phosphocreatine depletion ratio (4) was refitted excluding the parameter \( a_0 \), i.e.
APPENDIX F: Modelling Phosphocreatine resynthesis

\[
PDR = \frac{R-PCr(t)}{R} = \exp(-a \cdot t^b).
\]  

(5)

As before, by taking natural logarithms of the ratio PDR, we obtain the following power function relationship between \( \ln(PDR) \) and \( t \),

\[
\ln(PDR) = -a \cdot t^b.
\]  

(6)

Acknowledging that the ratio PDR will always be less than unity and, as such, the parameter 'a' will always be negative, by taking natural logarithms of the positive component of equation (6), i.e.,

\[
\ln(-\ln(PDR)) = \ln(a) + b \cdot \ln(t),
\]  

(7)

the parameters 'a' and 'b' can be fitted separately for each subject using linear least-squares regression. As can be seen in Fig. F2, the relationship between \( \ln(-\ln(PDR)) \) and \( \ln(t) \) is acceptably linear. Each subject's estimated parameters, \( r^2 \) and half recovery times are given in Table F1.

Compared with model (4), the quality of fit remained almost unchanged with five of the subjects' coefficients of determination \( (r^2) \) greater than or equal to 99% and the remaining three subjects' \( r^2 \) 98%, 98% and 94%.

Using the methods of Nevill et al. (1992), analysis of variance (ANOVA) can be used to test for the homogeneity of subjects' regression line parameters. A significant difference was found between the subjects' slope

---

**Fig. F2.** Double logarithmic-transformed phosphocreatine depletion ratio, \( \ln(-\ln(PDR)) \), by logarithmic-transformed recovery time, \( \ln(t) \), for all eight subjects.
Table F1. Each subjects' estimated parameters, \( r^2 \) and half recovery times for the phosphocreatine depletion ratio model (equation 7).

<table>
<thead>
<tr>
<th>Subject number</th>
<th>'a'</th>
<th>'b'</th>
<th>( r^2 ) (%)</th>
<th>Half recovery time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.054</td>
<td>0.62</td>
<td>98</td>
<td>62.6</td>
</tr>
<tr>
<td>2</td>
<td>0.076</td>
<td>0.50</td>
<td>94</td>
<td>82.0</td>
</tr>
<tr>
<td>3</td>
<td>0.055</td>
<td>0.58</td>
<td>99</td>
<td>80.0</td>
</tr>
<tr>
<td>4</td>
<td>0.083</td>
<td>0.66</td>
<td>98</td>
<td>25.3</td>
</tr>
<tr>
<td>5</td>
<td>0.036</td>
<td>0.80</td>
<td>100</td>
<td>42.0</td>
</tr>
<tr>
<td>6</td>
<td>0.102</td>
<td>0.52</td>
<td>100</td>
<td>38.9</td>
</tr>
<tr>
<td>7</td>
<td>0.080</td>
<td>0.51</td>
<td>99</td>
<td>71.2</td>
</tr>
<tr>
<td>8</td>
<td>0.095</td>
<td>0.51</td>
<td>99</td>
<td>51.0</td>
</tr>
</tbody>
</table>

(shape) and intercept (location) parameters 'b' and 'a' of model (7) (see Table F2). This finding suggests that each subjects' phosphocreatine resynthesis model is sufficiently different to preclude a common group resynthesis model. By fitting model (7) separately to each subject, the explained variance in the transformed phosphocreatine depletion ratio can be obtained (see Table F2) as \( R^2 = (26.71 - 0.39) / 26.71 = 98.5\% \).

Table F2. ANOVA to compare the subjects' phosphocreatine depletion ratio model (equation 7) parameters.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall regression</td>
<td>23.59</td>
<td>1</td>
<td>23.59</td>
<td>982.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Difference in intercepts</td>
<td>2.09</td>
<td>7</td>
<td>0.299</td>
<td>12.46</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Difference in slopes</td>
<td>0.64</td>
<td>7</td>
<td>0.091</td>
<td>3.79</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual error</td>
<td>0.39</td>
<td>16</td>
<td>0.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>26.71</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

By rearranging equation (5), the model for phosphocreatine resynthesis becomes,

\[
PCr(t) = R - R \exp(-at^b)
\]

as described in equation (1) in Chapter IV.
APPENDIX G: Respiratory parameters during recovery from sprinting

APPENDIX G

Respiratory responses during recovery from a 30 s sprint

INTRODUCTION

The contribution of aerobic energy sources to a single 30 s sprint has been reported to be \( \approx 23-28 \% \) of the total energy supply (Serresse et al., 1988; Withers et al., 1991), or even as high as \( 40 \% \) of the total energy (Medbo and Tabata, 1989, 1993) when cycling at 90 revs·min\(^{-1}\) (1.5 Hz) until exhaustion (34 s).

As seen in Chapters V and VI, when sprint exercise is repeated after a short recovery interval, aerobic metabolism (reflected in oxygen consumption, \( \dot{V}O_2 \)), is increased in comparison with the first exercise bout. However, when calculations for the percentage energy contribution are performed using the \( \dot{V}O_2 \) during a sprint, the oxygen uptake just before that sprint (baseline) must be subtracted.

The purpose of this study was to follow oxygen uptake and related cardiorespiratory parameters from the end of the standardised warm-up until 4 min after a maximal 30 s sprint. This provided a reference base for correcting \( \dot{V}O_2 \) measurements during repeated sprints on the cycle ergometer.

METHODS

Eight healthy male University students (body mass: 73.7±2.1 kg) volunteered to participate in this study. Five of the subjects had taken part in the biopsy studies described in this thesis. All subjects were thoroughly familiarised with the testing procedures and protocol, and reported to the laboratory after at least 4 hours of fasting.

Following 30 min of rest, subjects performed the standardised warm-up on the cycle ergometer (see Chapter III), and after a further 5 min of rest, a maximal 30 s sprint was performed. Power output was measured as described in Chapter III.
APPENDIX G: Respiratory parameters during recovery from sprinting

Fig. G.1. Schematic representation of the experimental protocol.

The subjects then remained seated on the cycle ergometer for 4 min. Expired air samples were collected in Douglas bags before, during and after the 30 s sprint as shown in Fig. G.1. Expired air samples were analysed as described in CHAPTER III. Heart rate was monitored every min, throughout the test using short range telemetry (Sports Tester, PE3000). Statistical analysis was performed using one-way analysis of variance for repeated measures, together with a Tukey post-hoc test, whenever necessary.

RESULTS

Power output and pedal speed parameters during the 30 s sprint are presented in Table G.1. The oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) in l·min$^{-1}$ and ml·kg$^{-1}$·min$^{-1}$, minute ventilation ($\dot{V}E$), respiratory equivalent for oxygen ($\dot{V}E \cdot \dot{V}O_2^{-1}$) and respiratory exchange ratio (R) are shown in Table G.2. The heart rate during the test is shown in Fig. G.2.
APPENDIX G: Respiratory parameters during recovery from sprinting

Table G.1. Peak power output (PPO), mean power output during the first 6 s (MP06), the first 10 s (MP010) and the 30 s of sprinting (MP030), minimum power output (MinP), fatigue index (FI), work done during acceleration of the flywheel to peak speed (Wacc), maximum pedal speed (MaxSp) and mean pedal speed for the 30 s (Sp30).

<table>
<thead>
<tr>
<th></th>
<th>PPO (W)</th>
<th>MP06 (W)</th>
<th>MP010 (W)</th>
<th>MP030 (W)</th>
<th>MinP (W)</th>
<th>FI (%)</th>
<th>Wacc (J)</th>
<th>MaxSp (revs.min⁻¹)</th>
<th>Sp30 (revs.min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>861</td>
<td>714</td>
<td>673</td>
<td>563</td>
<td>431</td>
<td>50</td>
<td>534</td>
<td>140</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>884</td>
<td>684</td>
<td>632</td>
<td>496</td>
<td>366</td>
<td>59</td>
<td>519</td>
<td>137</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>915</td>
<td>759</td>
<td>731</td>
<td>597</td>
<td>429</td>
<td>53</td>
<td>510</td>
<td>129</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>1247</td>
<td>948</td>
<td>841</td>
<td>606</td>
<td>348</td>
<td>72</td>
<td>843</td>
<td>163</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>978</td>
<td>705</td>
<td>640</td>
<td>501</td>
<td>359</td>
<td>63</td>
<td>714</td>
<td>145</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>1273</td>
<td>965</td>
<td>873</td>
<td>639</td>
<td>418</td>
<td>67</td>
<td>967</td>
<td>171</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>1197</td>
<td>861</td>
<td>799</td>
<td>634</td>
<td>449</td>
<td>62</td>
<td>645</td>
<td>145</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>874</td>
<td>732</td>
<td>690</td>
<td>567</td>
<td>424</td>
<td>51</td>
<td>473</td>
<td>129</td>
<td>101</td>
<td></td>
</tr>
</tbody>
</table>

\(\bar{x}\) 1029 796 735 575 403 60 651 145 108
SD 179 113 92 55 39 8 179 15 5
SE 63 40 33 19 14 3 63 5 2

Table G.2. Oxygen uptake \(\left<VO_2\right>\), carbon dioxide output \(\left<CO_2\right>\), ventilation \(\left<E\right>\), respiratory equivalent for oxygen \(\left<E \cdot VO_2^{-1}\right>\) and respiratory exchange ratio (R) before, during and after the 30 s sprint.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Sprint</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\left&lt;VO_2\right&gt;) (l·min⁻¹)</td>
<td>0.55 ±0.05</td>
<td>2.32 ±0.11</td>
<td>2.87 ±0.11</td>
</tr>
<tr>
<td>(\left&lt;CO_2\right&gt;) (ml·kg⁻¹·min⁻¹)</td>
<td>7.47 ±0.73</td>
<td>31.5 ±1.50</td>
<td>39.17 ±2.01</td>
</tr>
<tr>
<td>(\left&lt;E\right&gt;) (l·min⁻¹)</td>
<td>0.83 ±0.13</td>
<td>3.09 ±0.21</td>
<td>4.17 ±0.20</td>
</tr>
<tr>
<td>(\left&lt;E \cdot VO_2^{-1}\right&gt;)</td>
<td>11.30 ±1.83</td>
<td>41.94 ±2.88</td>
<td>56.85 ±3.11</td>
</tr>
<tr>
<td>R</td>
<td>26.8 ±4.7</td>
<td>108.8 ±18.2</td>
<td>102.5 ±5.2</td>
</tr>
</tbody>
</table>

Pre, before sprint; Only not significant differences indicated: *, from Pre; #, from Sprint; †, from 30"; ‡, from 2 min; §, from 3 min; all other differences are \(P<0.01\).
DISCUSSION

The main finding of this study was that all respiratory parameters (VO₂, VCO₂, VE, VE·VO₂⁻¹ and R) decreased to pre-sprint levels after only 4 min of recovery from the 30 s sprint. This finding is in agreement with Kowalchuk et al. (1988) who reported half times of 47±4 s and 105±15 s for VO₂ and VCO₂ decrease, respectively. The pre-sprint respiratory parameters are, however, elevated compared with resting values (Kowalchuk et al., 1988). The high R values (Table G.2) as a result of rapid CO₂ efflux from the muscle, indicate the important role of the lungs in the regulation of acid-base balance. The higher oxygen uptake measured during the initial 30 s after the sprint does not mean that VO₂ was indeed higher after the sprint. The highest VO₂ during a sprint is almost invariably achieved at the last seconds (Withers et al., 1991). The results of the present study probably reflect the “averaging effect” of the Douglas bag method. Due to the slow O₂ kinetics during the initial part of the sprint, the average VO₂ during the 30 s of sprinting is lower than the average VO₂ during the first 30 s of recovery.
APPENDIX G: Respiratory parameters during recovery from sprinting G.5

In summary, this study has shown that there is no significant difference between the oxygen uptake before a single 30 s sprint and that during the 4th min of recovery from the sprint. To improve the accuracy of calculations for aerobic energy contribution to a single 30 s sprint, the value of 0.55 l·min⁻¹ must be subtracted from the \( \dot{V}O_2 \) during the sprint.

When a 30 s sprint is repeated after 4 min of passive recovery, 0.78 l·min⁻¹ must be subtracted from the \( \dot{V}O_2 \) during that sprint. However, an extra oxygen consumption must be added to both sprints because muscle initially uses the oxygen stored in the myoglobin, and the haemoglobin of the blood perfusing it. This oxygen is about 1.5-2 mmol·kg dry muscle⁻¹ (Harris et al., 1975; Blei et al., 1993) and can be used to regenerate a total of 9-12 mmol ATP·kg dry muscle⁻¹ (assuming a P/O₂ ratio of 6.5).