HUMAN MUSCLE METABOLISM DURING INTERMITTENT MAXIMAL EXERCISE

by

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This thesis is dedicated to my parents,
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ABSTRACT

Many daily activities and sporting events require an individual to perform brief periods of maximal exercise (i.e. <10s) interrupted by limited periods of recovery. In the first study, an intermittent maximal exercise test was used to examine the decline in power output with successive exercise periods and identify the changes in the metabolic environment associated with such exercise. The exercise protocol consisted of ten 6s maximal sprints with 30s recovery between each sprint on a cycle ergometer. The results suggest that phosphocreatine (PCr) and anaerobic glycolysis provided the majority of the energy to sustain an average power output (MPO) of 870.1 $\pm$ 159.2W in the first sprint. In the final sprint, however, no change in lactate concentration was apparent, yet the average power output was still 73% of that in the initial sprint. It was suggested that the energy was derived from PCr degradation and oxidative metabolism.

In face of an apparent reduced contribution from glycolysis the possibility that accumulation of hydrogen ions within the muscle, and the associated acidosis, could partly account for a reduced glycolytic flux was investigated. In this study subjects were required to perform a given amount of work in each sprint after ingesting solution of either sodium bicarbonate ($0.3g.kg^{-1}$) or sodium chloride (1.5g in total), 2.25 hours prior to exercise. The time required to complete each exercise bout was recorded in both conditions and when it exceeded 10 seconds the test was terminated. Resting muscle pH (7.11 $\pm$ 0.04) and buffering capacity (24.05 $\pm$ 1.83mmol $H.^{+}kg^{-1}$ wm, over pH range 7.1-6.5) were not altered as a result of induced alkalosis. In addition, no differences were found in time taken to complete each sprint and at the end of the test muscle pH (6.77 $\pm$ 0.11) and all the glycolytic intermediates reached similar values in both conditions, despite the observed changes in acid-base balance of the blood in the alkalotic condition. There was a reduction in the catecholamine response to exercise during the alkalotic condition compared with the placebo condition. It was suggested that acidosis was not the primary cause of fatigue during maximal intermittent exercise.

The possibility that the decrease in PCr concentration could be a more contributory factor to fatigue during maximal intermittent exercise was also investigated. Subjects performed the same exercise test as in the first study, on three different occasions. On one occasion (A) the circulation of both legs was occluded during each recovery interval using two thigh cuffs. On a separate occasion (B) the cuffs were inflated for the first seven sprints after which the test was continued with full circulation. Finally, the control condition (C) was performed with the circulation intact. In the final sprint MPO was 80.5% and 53% of that achieved in the initial sprints in condition (C) and (A), respectively. The greater fatigue in (A) was not accompanied by greater acid-base changes in the blood as indicated by the blood pH and blood lactate concentrations in the post-exercise period. In condition (B) MPO was 60% of initial after seven sprints but recovered rapidly to 80% of initial in the final sprint. It was suggested that performance of intermittent maximal exercise was mainly dependent on PCr resynthesis.

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1. INTRODUCTION

A unique property of skeletal muscle is the ability to accelerate the transformation of chemical energy into mechanical work during the transition from rest to exercise. The rate at which energy is converted from one form to another determines the exercise intensity which can be tolerated. In bursts of all-out exercise, such as sprint running or cycling, man reaches his maximal rate of energy output within a few seconds. It is a common experience however, that as exercise continues performance begins to decline, giving rise to the phenomenon known as fatigue.

The aetiology of the fatigue experienced during such maximal effort continues to be the subject of controversy among scientists. Indeed, when one considers the ways in which human skeletal muscle may fail to generate the required power output, it ›es seem cl that it is difficult to identify any single factor responsible for the resulting decrease in performance. For instance, fatigue may be the result of central nervous command or peripheral mechanisms located within the contracting muscle.

The reintroduction of the Dunchenne needle biopsy technique (Bergstrom, 1962) in conjunction with improved methods of biochemical analysis and the development of instrumented ergometers which monitor the time course of fatigue (Bar-Or, 1978; McCartney, Heigenhauser and Jones, 1983a; Lakomy, 1986; Lakomy, 1988), have increased our understanding of the metabolic events associated with the phenomenon of fatigue. However, the further implication that a causal relationship exists between metabolic changes and fatigue is far from certain. Even if fatigue during all-out exercise is metabolic in origin, the mechanism and site(s) where this might occur are not known. Owing to the extremely high rates of energy turnover that are required to sustain this type of exercise, fatigue has been considered to result from a mismatching between the rate at which chemical energy is utilised and the rate at which is produced in skeletal muscles. This imbalance between the rates of energy demand and supply may primarily arise either from an insufficient energy provision of the metabolic machinery to meet the

energy needs of the contracting muscle or from a reduction of the contractile activity itself, thus reducing demand and hence energy provision.

In the past two decades, a relatively large number of studies have examined the metabolic responses to continuous short-term dynamic exercise that requires an all-out effort. However, information about intermittent exercise involving brief periods (i.e. <10s) of maximal intensity accompanied by short rest periods is still sparse. The importance for investigation of muscle metabolism and fatigue during this type of exercise, can be justified primarily on the grounds that a large number of the population participates in recreational sports, such as soccer, squash, hockey, rugby and basketball, which require bursts of high-intensity exercise. Indeed, arising from the increased opportunities available through improved provision of recreational facilities and from the marked change in public attitudes towards physical activity as pleasurable and healthy, the so called 'multiple-sprint sports' (Williams, 1987) have recently become the most popular participation sports. In addition, an understanding of the factors that limit physical performance during intermittent all-out exercise could further contribute to promoting excellence in such sports through the construction of sound training programmes.

The aim of the studies undertaken in this thesis, was to identify the metabolic responses to intermittent maximal exercise in order to examine the relationship between muscle metabolism and fatigue. A cycle ergometer was modified for this purpose (Lakomy, 1988), for the laboratory examination of sprint performance during this type of exercise. The exercise tests consist of repeated maximal sprints of brief duration (i.e. <10s) on a cycle ergometer with short recovery intervals between sprints. Previous work (Wooton and Williams, 1983) has shown that power output during brief multiple cycle ergometer sprinting is impaired when the recovery duration between bouts is short (30s).

The thesis is presented as follows: The review of literature is concerned with the metabolic factors which are associated with the onset of fatigue during short term high-intensity exercise. This is followed by a methodology chapter which describes the instrumentation of the cycle ergometer used for measurement of power output, the administration of the exercise-tests employed and the biochemical techniques used for the analysis of the blood and muscle samples obtained. The experimental studies undertaken are presented in the order in which they were completed.

The purpose of the first study (Chapter 4) was to describe the metabolic changes in muscle and blood of subjects performing intermittent maximal exercise and examine their relationship with performance.

The study reported in Chapter 5 examined the influence of sodium bicarbonate administration on intermittent exercise performance. Using this particular approach the purpose was to establish whether or not changes in performance were accompanied by favourable changes in blood and muscle pH and muscle metabolism.

In the light of the findings of the previous two studies, the final study (Chapter 6) was designed to examine the effect of circulatory occlusion, of the legs, on performance during intermittent exercise of maximal intensity. The discussion section in this chapter therefore, draws together the findings of all studies and focuses attention on the question of possible mechanisms underlying the cause of fatigue during intermittent maximal exercise.

2. REVIEW OF LITERATURE

2.1 INTRODUCTION

Exercise performed at an intensity greater than that required to elicit maximal oxygen uptake, can only be tolerated for a short period of time before fatigue necessitates termination of the activity. In the past two decades, several studies using a variety of exercise models, have examined the metabolic changes in muscle and blood during such exercise in order to investigate the relationship between muscle metabolism and fatigue.

The focus of attention in this chapter is a consideration of our current understanding of the metabolic factors associated with the onset of fatigue during short-term exercise. In doing so, the regulatory mechanisms of energy production and utilisation that are thought to be involved during this type of exercise are discussed. Emphasis is also placed on the accumulation of end products of glycolysis and in particular on the accumulation of protons and the resultant decrease in intramuscular pH. Muscle metabolism during the early phase of recovery from short term exercise is also examined because this provides an alternative approach to the study of fatigue when a second bout of exercise is performed. Finally, the possible mechanisms by which metabolic changes can cause the onset of fatigue are discussed. It should be noted, however, that no attempt is made to provide a simple answer to what is a complex phenomenon. The data from the different protocols used in the literature are interpreted with caution, since the mechanism of fatigue is likely to depend on the type and intensity of the activity.

In referring to the work of other authors a clarification of the terms used to describe the intensity of exercise is required. The term 'maximal exercise' is used to describe exercise which is performed at the greatest rate throughout the exercise period. The term 'high-intensity exercise' is used to describe all other exercise intensities

that are greater to that required to elicit maximal oxygen uptake. In this review, concentrations of muscle metabolites are expressed as mmol.kg^{-1} dry muscle.

2.2 MEASUREMENT OF MECHANICAL POWER OUTPUT GENERATED DURING MAXIMAL EXERCISE

Skeletal muscle is characterised functionally by its force-velocity and fatigue characteristics. In vitro, the relationship between force and velocity is hyperbolic (Hill, 1938) and fatigue is manifested as the decline in force development that occurs with repeated contractions at constant muscle stimulation rates (Edwards, 1981). During maximal dynamic exercise the force-velocity and fatigue characteristics of human skeletal muscle cannot be established in the same way as in isolated muscle preparations. This is because this type of exercise requires poly-articular movements involving large muscle groups. In this situation an alternative approach is the measurement of mechanical power output during exercise. The power produced using dynamic muscular contractions depends on both force and velocity factors.

The measurement of human power in exercise requires the determination of the rate at which the energy, produced within the body, is dissipated into the external load. Thus, the power in this context is defined as the time-rate of doing work or work accomplished per unit time. The limitation to measurement of power output generated during short-term maximal dynamic exercise has been one of methodology. This is because the maximal rate at which work can be performed, decreases in a non-linear manner as the duration of the exercise increases (Fletcher, 1964). Therefore, any device measuring performance during maximal exercise, must be sensitive to rapid changes in power output.

In the past, the maximal power generated over a brief period of time has been calculated only from measurements made during vertical jumping (Sargent, 1921; Sargent, 1924) and stair climbing (Margaria, Aghemo and Rovelli, 1966). In the 1970's, the development of a simple laboratory test by Bar-Or (1978) using a friction-loaded cycle ergometer, allowed maximal power output to be measured and the time course of the decline of power output to be monitored. This test which has become known as the 'Wingate Test' requires the subject to cycle at maximum speed against a predetermined resistance related to body weight for 30s.

The relatively low cost of instrumenting the friction-loaded cycle ergometers, already in use in exercise physiology laboratories, contributed to the ready acceptance of the Wingate Test as a measure of the power output generated during maximal exercise of short duration. However, until recently, power output was conventionally determined from the product of flywheel revolutions, performed in a fixed-time interval (usually 3-10s), and resistive load. As the assessment of true maximal power output requires measurement of instantaneous values of force and velocity, this condition, in most cases has not been satisfied and instead the mean power (work divided by time) has been measured. In addition, conventional methods have ignored the moment of inertia of the flywheel and have assumed that the flywheel revolves at a constant angular velocity. Consequently, the amount of work done has been calculated ignoring any acceleration involved. The combination of errors resulting from making these assumptions may explain why some recent studies have found a large difference between instantaneous power corrected for flywheel acceleration and uncorrected power output (Lakomy, 1988).

The measurement of power output on a friction-loaded cycle ergometer during maximal exercise has been criticised on the grounds that this method employs accelerating movements in which the muscles work at an optimal velocity of shortening for only a brief time (McCartney, Heigenhauser, Sargeant and Jones, 1983). This means that if the rate of pedalling is increased above this optimum value (90-110rpm) (McCartney et al, 1983) then a portion of the decline in power output may be attributed to the force-velocity relationship of muscle contraction. Indeed, it has been shown that the development of maximal power output on a friction-loaded cycle ergometer is attained before maximal speed is reached (Lakomy, 1988). Nevertheless, the inability to maintain power output during successive maximal exercise periods of brief duration (i.e. 6s) cannot be attributed to the force-velocity relationship of muscle contraction. It has been shown that during six 6s sprints with 30s recovery intervals, power output was gradually decreased although subjects were able to achieve optimum pedalling speeds in all exercise periods (Lakomy, 1988).

2.3 ENERGY METABOLISM IN SKELETAL MUSCLE DURING HIGH-INTENSITY EXERCISE

ATP utilisation during muscular contraction

Skeletal muscle is an extreme case of a tissue that is specialised for the rapid provision of energy. During exercise which demands maximal levels of power output, energy expenditure may increase by as much as 1000-fold that of rest and this can be achieved within fractions of a second (Hultman and Sjöholm, 1983b).

Muscle contraction relies on a series of chemical reactions that require an input of free energy. The immediate source of this energy derives from the hydrolysis of a specific chemical compound, called adenosine 5'-triphosphate (ATP). When muscle contracts ATP is hydrolised to adenosine 5'-diphosphate (ADP) and inorganic phosphate (Pi) and the stored energy in the ATP molecule is liberated by the typical exergonic reaction:

$$ATP + H_2O \rightarrow ADP + Pi + \text{energy } (7 \text{ kcal.mole}^{-1})$$

In a contraction-relaxation cycle, there are three different ATP splitting reactions catalysed through the activity of three major ATPases; actomyosin ATPase responsible for formation and breaking of cross-bridges, resulting in shortening of the muscle; calcium transporting ATPase (Ca^{2+} ATPase) responsible for re-uptake and thus, decrease of cytosolic Ca^{2+} concentration for relaxation; and, sodium-potassium ATPase (Na^+-K^+ ATPase) responsible for restoring the ionic balance in the sarcolemma and T-tubule system membranes, after each action potential.

The rate of energy demand by the muscle is determined by the rate of ATP degradation through the activity of ATPases in the contracting muscle. The contribution to total energy consumption (total ATP turnover) by each of these processes has been proven difficult to determine. It appears however, that the major fraction of the ATP turnover, at least at the start of contraction, is utilised specifically in the actomyosin ATPase reaction (Hultman, Spriet and Söderlund, 1987). During electrical stimulation of the quadriceps femoris muscle of men at near

maximum contraction force (50Hz), it is estimated that the ATP turnover rate utilised in the actomyosin ATPase reactions alone is 8.6mmol ATP.Kg^{-1} dry muscle (d.m).s^{-1} at the start of contraction (1.28s of stimulation) (Hultman et al, 1987) and the energy cost for Ca^{2+}-cycling and electrolyte transport increases the total ATP turnover rate to about 11mmol ATP.Kg^{-1}dm.s^{-1} (Hultman and Sjöholm, 1983b). Although it is not known whether the percentage contribution of these processes varies with the stimulation frequency, during continuous contraction, the total ATP turnover rate does (Hultman and Sjöholm, 1983b). Recently, by comparison of stimulations producing the same amount of isometric work but with a different number of contractions, it has been estimated that the energy cost for activation and relaxation of a one second tetanic contraction is approximately 37% of the total ATP production. However, these estimates did not include any Ca^{2+} pumping occurring during the tetanus (Bergström and Hultman, 1988). Furthermore, the energy cost during several intermittent isometric contractions has been found to be significantly higher than the energy utilisation associated with one continuous contraction of the same duration (Chasiotis, Bergström and Hultman, 1987; Spriet, Söderlund and Hultman, 1988; Bergström and Hultman, 1988). Collectively, in these studies it has been suggested that the increased ATP utilisation of intermittent contractions is related to enhanced Ca^{2+} transport ATPase activity during relaxation and enhanced actomyosin ATPase activity during the early part of each contraction.

Estimates of the anaerobic ATP turnover rate during dynamic exercise also vary depending on the intensity and duration of exercise (Table 2.1). A value of 10.5mmol ATP.Kg^{-1}dm.s^{-1} is estimated during 6s of maximal cycling (Boobis, Williams and Wootton, 1982) and an even higher value of 17.3mmol ATP.Kg^{-1}dm.s^{-1} is estimated (possibly over-estimated) during 10s of maximal isokinetic cycling at 140rpm (Jones, McCartney, Graham, Spriet, Kowalchuk, Heigenhauser and Sutton, 1985). During maximal cycling or sprint running lasting 30s the ATP turnover rate in the human quadriceps femoris muscle is of the order of 6-10mmol ATP.Kg^{-1}dm.s^{-1}.

TABLE 2.1 Estimates of anaerobic ATP turnover rate in human quadriceps femoris muscle during high-intensity exercise and percentage contribution to ATP resynthesis from the degradation of PCr and anaerobic glycolysis.

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	ATP TURNOVER RATE ($mmol.kg^{-1}dm.s^{-1}$)	% CONTRIBUTION PCr	Glycolysis	ATP
	CYCLING						
Boobis et al (1982)	max	30	4M	6.3*	29.9	64.4	5.7
	($75g.kg^{-1}bw$)	6	4M	10.5*	46.8	53.0	-
Jacobs et al (1982)	max ($75g.kg^{-1}bw$)	30	9F	4.1*	30.8	63.3	5.9
Jones et al (1985)	Isokinetic max (60rpm)	30	5M	9.7*	17.7	79.0	3.2
		10	2M	16.6*	37.5	59.0	3.5
	Isokinetic max (140rpm)	30	5M	8.0*	11.0	89.0	-
		10	2M	17.3*	31.3	64.3	4.4
McCartney et al (1986)	Isokinetic max (100rpm)	30 (1st sprint)	4M	7.6*	18.8	77.3	3.9
Spriet et al (1989)	Isokinetic max intermittent 4min recovery (100rpm)	30 (2nd sprint)	8M+F	2.5*	26.1	71.5	2.4
		30 (3rd sprint)		1.9*	35.4	61.4	3.2

Note:
*ATP turnover rates calculated by the present author and considered to be equal to the sum of ΔPCr + ΔATP + 1.5 x Δlactate + 1.5 x Δpyruvate as previously described (Sahlin and Henriksson, 1984); M refers to males and F to females.

TABLE 2.1 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	ATP TURNOVER RATE ($mmol.kg^{-1}dm.s^{-1}$)	% CONTRIBUTION PCr	Glycolysis	ATP
	RUNNING						
Cheetham et al (1986)	max	30	8F	6.13*	31.7	62.9	5.5
Nevill et al (1989)	max	30	16M+F	6.2*	30.0	66.6	3.4
	VOLUNTARY ISOMETRIC						
Sahlin & Ren (1989)	Contraction - 1	66% - 50% MVC	5M	-	36.0	61.0	-
	2 (2min after 1)			-	50.0	45.0	-
	3 (4min after 1)			-	41.0	58.0	-
	ELECTRICAL STIMULATION						
Hultman & Sjoholm (1983a)	20Hz (50 - 75% MVC)	first 10	9M+F	5.6	60.0	40.0	-
		last 10 (total 50s)		4.0	8.0	92.0	-
Hultman & Sjoholm (1983b)	50Hz	1.28		11.0	80.0	20.0	-
	20Hz	3.2		7.8	7.0	30.0	-
Spriet et al (1987a)	Intermittent 20Hz (22% MVC) (1.6s stimul-1.6s recovery)	0-25.6	7M	6.12	40.0	58.0	2
		25.6-51.2		2.56	-	83.0	-
		51.2-76.8		2.19	-	90.0	-
		76.8-102.4		0.64	-	92.0	-

TABLE 2.1 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	ATP TURNOVER RATE ($mmol.kg^{-1}dm.s^{-1}$)	% CONTRIBUTION PCr	Glycolysis	ATP
Spriet et al (1988)	Intermittent 20Hz						
	(25% MVC)	0-51.2	12M	4.5	30.0	65.0	5
	(1.6s stimul-1.6s recovery)	51.2-102.4		1.6	13.0	85.0	2
	Continuous 20Hz	0-51.2		3.8	30.0	65.0	5
		51.2-102.4		1.4	14.0	85.0	1
Bergstrom & Hultman (1988)	Intermittent 20Hz						
	(25% MVC)	0-22	8M+F	6.6	-	-	-
	(0.8s work - 0.8s recovery)	22-51		4.3	-	-	-
	Intermittent 20Hz	0-22		5.5	-	-	-
	(3.2s work - 3.2s recovery)	22-51		3.9	-	-	-
Ren et al (1988)	2 Contractions 20Hz						
	(70% MVC)	10(1)	10M	5.54	57.0	40.0	3
	Recovery 60s-occlusion	10(2)		5.73	43.0	54.0	3
	2 Contractions 20Hz						
	(70% MVC)	10(1)	10M	5.62	-	-	-
	Recovery 60s-intact circul.	10(2)		5.76	-	-	-

Note: In all isometric studies circulation to the thighs has been occluded.

These high rates of ATP utilisation required to support intense dynamic or isometric exercise of short duration are thus, largely dependent on the muscle's ability to regenerate ATP anaerobically.

Changes in ATP content during muscular contraction

In resting human skeletal muscle the concentration of ATP determined in acid extracts of biopsy samples is approximately 24.0 $\pm$ 2.6mmol.Kg^{-1}dm, accounting for 87.6% of the total adenine nucleotide store (TAN[1]) (Harris, Hultman and Nordesjö, 1974). Several studies have reported relatively small changes in ATP concentration following intense exercise. Typically, the decline in ATP content observed after voluntary short-term maximal exercise or in response to electrical stimulation at near maximal contraction force never exceeds 45% of the resting value (Table 2.2). It is possible, therefore, that only a proportion of the ATP in muscle is available as an energy source for muscle contraction per se, which would last for only a few contractions during maximal exercise.

The observed decline in ATP concentration during intense exercise is not accompanied by a stoichiometric increase in ADP and/or AMP concentrations within the muscle. Instead, there is a loss of total adenine nucleotides and a stoichiometric production of inosine-monophosphate (IMP) (Sahlin, Palmskog and Hultman, 1978; Harris and Hultman, 1985). This production of IMP occurs via the enzymatic action of AMP-deaninase and is thought to be beneficial to the muscle cell by regulating the relative concentrations of ADP and AMP in order to keep the energy charge of the cell high (Sahlin et al, 1978). This is achieved by promoting continued formation of ATP from ADP via the adenylate-kinase reaction ($2ADP \rightleftharpoons ATP + AMP$).

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1. TAN = ATP + ADP + AMP
   AMP = adenosine 5'-monophosphate

TABLE 2.2 Changes in muscle ATP content ($mmol.kg^{-1}dm$) during high-intensity exercise.

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE ATP Values	POST-EXERCISE ATP Values	% DECREASE
	CYCLING					
Boobis et al (1982)	max	30	4M	24.4	13.7	43.9
		6		24.4	22.2	9.0
Jacobs et al (1982)	max	30	9F	20.9	13.8	34.0
Jones et al (1985)	Isokinetic max (60rpm)	30	5M	25.7	16.3	36.6
		10	2M	25.7	19.9	22.6
	Isokinetic max (140rpm)	30	5M	23.1	23.1	0.0?
		10	2M	23.1	15.5	32.9
McCartney et al (1986)	Isokinetic max (100rpm)	4 x 30 (4min intervals)	4M	22.4	13.9	40.0
Spriet et al (1989)	Isokinetic max (100rpm)	3 x 30 (4min intervals)	8M+F	-	15.5	-
	RUNNING					
Naveri et al (1978)	max	3 x 300m	5M	24.9	17.2	30.9
Cheetham et al (1986)	max	30	8F	28.2	17.9	37.0
Hirvonen et al (1987)	max	4.4 (40m)	7M	23.0	18.3	20.4
		6.6 (60m)		22.4	15.9	29.0
		8.8 (80m)		24.1	17.8	26.1
		11.5 (100m)		22.1	15.9	28.1
Nevill et al (1989)	max	30	16M+F	26.7	19.2	28.0

TABLE 2.2 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE ATP Values	POST-EXERCISE ATP Values	% DECREASE
	VOLUNTARY ISOMETRIC					
Sahlin & Ren (1989)	Contraction 1	66 - 50%	10M	29.2	25.6	12.3
	2 (2min after)	(MVC)	5M	27.6	25.4	8.0
	3 (4min after)		5M	27.3	25.4	7.0
	ELECTRICAL STIMULATION					
Hultman & Sjoholm (1983a)	20Hz (50 - 75% MVC)	50	9M+F	-	-	24.0
Spriet et al (1987a)	Intermittent 20Hz	25.6	7M	24.8	21.8	12.0
	(22% MVC)	51.2			17.3	30.2
	(1.6s stimul-1.6s	76.8			15.5	37.5
	recovery)	102.4			14.0	43.5
Chasiotis et al (1987)	Intermittent 20Hz	10.0	4M+F	26.0	23.2	2.8
	(25% MVC)	22.0			22.2	14.6
	(1.6s stimul-1.6s	54.4			16.4	37.0
	recovery)					
	Continuous 20Hz	12.0		26.0	25.4	2.0
	(25% MVC)	22.0			23.9	8.0
		52.0			19.6	24.6

As the content of ATP is never markedly reduced in fatigued human muscle to critically low levels consistent with rigor development (Sahlin, Edström, Sjöholm and Hultman, 1981), ATP must be regenerated at the same rate as it is utilised, if the rate at which external work can be performed is to be maintained. It seems therefore, more appropriate to regard ATP as a mediator of energy rather than as an energy source.

The potential capacity for energy provision (i.e. the amount of ATP that can be produced) through complete oxidation of carbohydrates and free fatty acids within the mitochondria (oxidative metabolism) is great, but the maximal rate at which ATP may be regenerated by these processes is relatively low to meet the demands of a few seconds of maximal exercise. Additionally, aerobic ATP production requires a relatively long time to reach its maximal potential due to the necessity of adjusting the oxygen transporting system (circulation and respiration) to the demands (Sahlin, 1986). Consequently, during maximal exercise, the muscle cell is dependent largely on the energy provision systems that result in the greatest rates of ATP resynthesis in a very short time. This is achieved anaerobically, through the degradation of creatine phosphate (PCr) and through glycogenolysis (glycogen breakdown) resulting in lactic acid formation. Both substrates, are located within the muscle cells in close contact with the contractile mechanism and can thus be utilised immediately in response to sudden large changes in energy demand.

ATP resynthesis from creatine phosphate degradation

The content of PCr is three to four times higher than that of ATP in resting human skeletal muscle determined in biopsy samples (Table 2.3) although, it has been argued on the basis of NMR data from animal and human muscle, that, there might be an under-estimation of resting concentration of PCr measured chemically in biopsy samples (Creshull, Dawson, Edwards, Gadian, Gordon, Radda, Shaw and Wilkie, 1981; Edwards, Dawson, Wilkie, Gordon and Shaw, 1982). In contrast to the relatively modest changes in ATP concentration, more pronounced falls of PCr have been reported during intense exercise (Table 2.3). Therefore, PCr has

TABLE 2.3 Changes in muscle PCr content (mmol.kg^{-1}dm) and rate of PCr degradation (mmol.kg^{-1}dm.s^{-1}) during high-intensity exercise.

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE PCr Values	POST-EXERCISE PCr Values (% decrease)	RATE OF PCr DEGRADATION
	CYCLING					
Boobis et al (1982)	max	30	4M	84.3	28.8 (65.8%)	1.9
		6		84.3	54.8 (35.0%)	4.9
Jacobs et al (1982)	max	30	9F	62.7	25.1 (60.0%)	1.3
Jones et al (1985)	Isokinetic max (60rpm)	30	5M	85.0	33.6 (60.4%)	1.7
		10	2M	85.0	22.7 (73.3%)	6.2
	Isokinetic max (140rpm)	30	5M	78.2	51.9 (33.6%)	0.9(?)
		10	2M	78.2	24.0 (69.3%)	5.4
McCartney et al (1986)	Isokinetic max (100rpm)	4 x 30 (4min intervals)	4M	61.3	2.6 (96.0%)	-
Spriet et al (1989)	Isokinetic max (100rpm)	3 x 30 (4min intervals)	8M+F	39.0 (S2)	19.8 (50.8%)	0.6
				36.9 (S3)	16.7 (54.7%)	0.7
	RUNNING					
Naveri et al (1978)	max	3 x 300m	5M	75.7	19.8 (73.8%)	-
Cheetham et al (1986)	max	30	8F	87.7	31.2 (64.4%)	1.9
Hirvonen et al (1987)	max	4.4 (40m)	7M	48.0	22.1 (54.0%)	5.9
		6.6 (60m)		45.4	20.2 (55.5%)	3.8
		8.8 (80m)		48.6	17.0 (65.0%)	3.6
		11.5 (100m)		44.9	15.1 (66.4%)	2.7
Nevill et al (1989)	max	30	16M+F	84.0	28.0 (66.7%)	1.9

Note: S2 = Sprint 2; S3 = Sprint 3

TABLE 2.3 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE PCr Values	POST-EXERCISE PCr Values (% decrease)	RATE OF PCr DEGRADATION
	VOLUNTARY ISOMETRIC					
Sahlin & Ren (1989)	Contraction 1	66 - 50%	10M	87.3	9.4 (89.0%)	1.5
	2 (2min after)	(MVC)	5M	57.8	11.3 (80.0%)	-
	3 (4min after)		5M	74.3	11.3 (85.0%)	-
	ELECTRICAL STIMULATION					
Hultman & Sjoholm (1983a)	20Hz (50 - 75% MVC)	50	9M+F	75.0	10.0 (86.7%)	1.3
Spriet et al (1987a)	Intermittent 20Hz	25.6	7M	78.7	15.7 (80.1%)	2.5
	(22% MVC)	51.2			9.0 (88.6%)	1.4
	(1.6s stimul-1.6s	76.8			5.4 (93.1%)	1.0
	recovery)	102.4			5.5 (93.1%)	1.0
Chasiotis et al (1987)	Intermittent 20Hz	10.0	4M+F	75.3	41.4 (45.0%)	3.4
	(25% MVC)	22.0			20.2 (73.2%)	2.5
	(1.6s stimul-1.6s recovery)	54.4			3.4 (95.5%)	1.4
	Continuous 20Hz	12.0		75.3	42.7 (43.3%)	2.7
	(25% MVC)	22.0			28.1 (62.7%)	2.2
		52.0			6.9 (90.8%)	1.3

TABLE 2.3 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE PCr Values	POST-EXERCISE PCr Values (% decrease)	RATE OF PCr DEGRADATION
Ren et al (1988)	2 Contractions 20Hz					
	(70% MVC)	10(1*)	10M	85.1	53.6 (37.0%)	3.2
	Recovery 60s-occlusion	10(2)		50.3	25.3 (49.7%)	2.5
	2 Contractions 20Hz					
	(70% MVC)	10(1)		79.3	49.4 (37.7%)	3.0
	Recovery 60s-intact circul.	10(2)		70.8	34.9 (50.7%)	3.6
McGilvery (1975)	Theoretical maximum	1				6.9-12.0

classically been considered to function as a storage of high-energy phosphate that buffers changes in ATP and ADP levels[1].

The rephosphorylation of ADP to ATP at the expense of PCr is mediated by the enzyme creatine kinase (CK), according to the reaction:

$$PCr + ADP + H^+ \rightleftharpoons ATP + creatine + Pi$$

At rest the creatine kinase reaction is at equilibrium and it has been postulated that this state also exists at the end of intense exercise inspite of dramatic changes in the PCr content (Sahlin, Harris and Hultman, 1975; Harris, Sahlin and Hultman, 1977). This has been attributed to the direct involvement of hydrogen ions (H^+) in the equilibrium of the reaction (Sahlin et al, 1975). If this is true, a decrease in intracellular pH would thus, cause a decrease of PCr levels either, directly, by lowering the equilibrium concentration of PCr or, indirectly, through increased ADP (Sahlin et al, 1975; Sahlin, 1978). It has been shown that during exhaustive exercise the decrease in PCr content proceeded curvilinearly with respect to the increase in muscle lactate and this relationship was independent of duration, intensity and type of performed exercise (Harris et al, 1977). This is indeed surprising, as the rate of energy demand and production should vary within broad limits with different exercise intensities.

Several studies have reported substantial PCr degradation of a magnitude of 80% or more of resting values following intense dynamic exercise (Hultman, Bergström and McLennan-Anderson, 1967; McCartney, Spriet, Heigenhauser, Kowalchuck, Sutton and Jones, 1986), sustained voluntary

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1. In apparent contrast to this view, it has been suggested that PCr and creatine, function as a shuttle for the transport of high energy phosphate between compartments of adenylates within muscle cells (i.e. from the mitochondrial to the myofibrillar ends in muscle) (Bessman and Geiger, 1981). This proposal is beyond the scope of this review.

isometric contractions (Sahlin and Ren, 1989) or electrical stimulation at near maximum contraction force (Hultman and Sjöholm, 1983a) lasting from 30s to 2min. Although, according to the theoretical calculations of Margaria, Cerretelli and Mangeli (1964) and Newsholme (1980), PCr stores could also be totally depleted in maximal exercise of 5-7s duration, this does not appear to be the case. Studies of brief maximal dynamic exercise (4.4-11s) have shown that the PCr content in biopsy samples of human quadriceps muscle is depleted by 60-70% of resting values (Boobis et al, 1982; Jones et al, 1985; Hirvonen, Rehumen, Rusko and Harkönen, 1987). What is evident, however, from these studies is that the greatest importance of PCr as an energy store is most probably during the very first few seconds of increased energy demand. This is supported by the higher rates of PCr degradation that are observed during brief maximal dynamic exercise compared to those reported during maximal exercise of durations lasting 30s or more (Table 2.3). This also holds true during maximal voluntary isometric contraction or during electrical stimulation. It has been reported that the initial rate of ATP production from PCr degradation during this type of exercise is about 8-9mmol ATP.Kg^{-1} dm.s^{-1} calculated over the first 3-4s, accounting for 60-80% of the total ATP turnover rate (Bergström, Harris, Hultman and Nordesjö, 1971; Hultman and Sjöholm, 1983b). Despite these high rates of PCr degradation, glycogenolysis must also be activated in the first few seconds of exercise to sustain the high rates of energy demand.

Glycogen degradation during high-intensity exercise

Skeletal muscle is a major site of glycogen storage. At rest, skeletal muscle glycogen concentrations range between 250 and 500mmol glucosyl units.Kg^{-1}dm (Table 2.4). Thus, the glycogen store in skeletal muscle is an energy provider, potentially much larger than the PCr store. Complete oxidation of glycogen results in the formation of 37 moles of ATP per glucosyl unit whereas glycogen degradation leading to lactate formation releases enough energy for the resynthesis of only 3 moles of ATP per glucosyl unit. Lactate formation is thus an extremely inefficient way of utilising the glycogen store of muscle but like PCr breakdown, it provides a relatively rapid supply of ATP. The processes

TABLE 2.4 Changes in muscle glucogen content (mmol.glucosyl units.kg^{-1}dm) and rate of glycogen degradation (mmol.glucosyl units.kg^{-1}dm.s^{-1}) during high-intensity exercise.

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE GLYCOGEN	POST-EXERCISE GLYCOGEN (% decrease)	RATE OF GLYCOGEN DEGRADATION
	CYCLING					
Gollnick et al (1982)	150% VO_2 max	60	6	568	456 (19.7%)	1.9
Boobis et al (1982)	max	30	4M	267	201 (24.5%)	2.2
		6		267	229 (14.2%)	6.3
Jacobs et al (1982)	max	30	9F	360	278 (22.8%)	2.7
King et al (1985)	Isokinetic max	45	10M	566	446 (21.2%)	2.7
McCartney et al (1986)	Isokinetic max (100rpm)	4 x 30 (4min recovery)	4M	369	291 (21.2%)	2.6 (Sprint 1)
				-	223 (39.6%)	- (Sprint 4)
Spriet et al (1989)	Isokinetic max (100rpm)	3 x 30 (4min recovery)	8M+F	319	271 (14.8%)	1.6 (Sprint 2)
				287	272 (5.3%)	0.5 (Sprint 3)
	RUNNING					
Naveri et al (1978)	max	3 x 300m	5M	208	160 (23.1%)	0.4
Cheetham et al (1986)	max	30	8F	281	212 (25.0%)	2.3
Nevill et al (1989)	max	30	16M+F	317	215 (32.0%)	3.4

TABLE 2.4 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE GLYCOGEN	POST-EXERCISE GLYCOGEN (% decrease)	RATE OF GLYCOGEN DEGRADATION
	ELECTRICAL STIMULATION					
Hultman & Spriet (1986)	Intermittent 20Hz (1.6s stimul-1.6s recovery)	80	7M+F	392	338 (14.0%)	0.7
Chasiotis et al (1987)	Intermittent 20Hz (25% MVC) (1.6s stimul-1.6s recovery)	10.0	4M+F	-	-	1.3
		22.0		-	-	1.5
		54.4		-	-	1.5
	Continuous 20Hz (25% MVC)	12.0		-	-	0.9
		22.0		-	-	1.2
		52.0		-	-	1.3

involved in the degradation of glycogen to lactate formation collectively constitute the anaerobic glycolytic pathway. These processes occur in the cytoplasm and are illustrated in Figure 2.1.

The primary reaction in this process is the phosphorylative degradation of glycogen to glucose-1-phosphate (G-1-P); the glycogenolysis. The enzyme that catalyses this reaction is known as glycogen phosphorylase. The activity of phosphorylase in skeletal muscle is very high and necessarily so, since glycogen must be degraded rapidly in order to produce energy at sufficient rate for muscle contraction during high-intensity exercise. Although free glucose in the muscle is also available for anaerobic metabolism, through the glycolytic pathway, glycogen seems to be preferred over glucose. This is so, because the intracellular content of free glucose in muscle is small, approximately 1.7mmol.Kg^{-1}dm (Harris et al, 1974) and penetration of glucose of the plasma membrane is a slow process compared to the rate of formation of hexose monophosphates from locally stored glycogen (about 1mmol.Kg^{-1}dm.min^{-1} compared to over 100mmol.Kg^{-1}dm.min^{-1}, respectively) (Hultman and Sjöholm, 1983b). Furthermore, the phosphorolytic cleavage of glycogen is energetically advantageous because the released sugar (G-1-P) is phosphorylated. In contrast, glucose would have to be phosphorylated at the expense of one ATP to enter the glycolytic pathway. An additional point is that G-1-P cannot diffuse out of the cell whereas glucose can (Stryer, 1986).

The reduction of NAD^+ in the glyceraldehyde-3-phosphate dehydrogenase reaction requires that the NADH which is produced, must be reoxidised so that redox balance (NADH/NAD^+ ratio) is maintained and intermediate metabolites and co-factors do not accumulate in their reduced forms. Under anaerobic conditions, in skeletal muscle, this is achieved by the lactate dehydrogenase reaction:

$$\text{pyruvate} + \text{NADH} + H^+ \overset{\text{LDH}}{\rightleftharpoons} NAD^+ + \text{lactate}$$

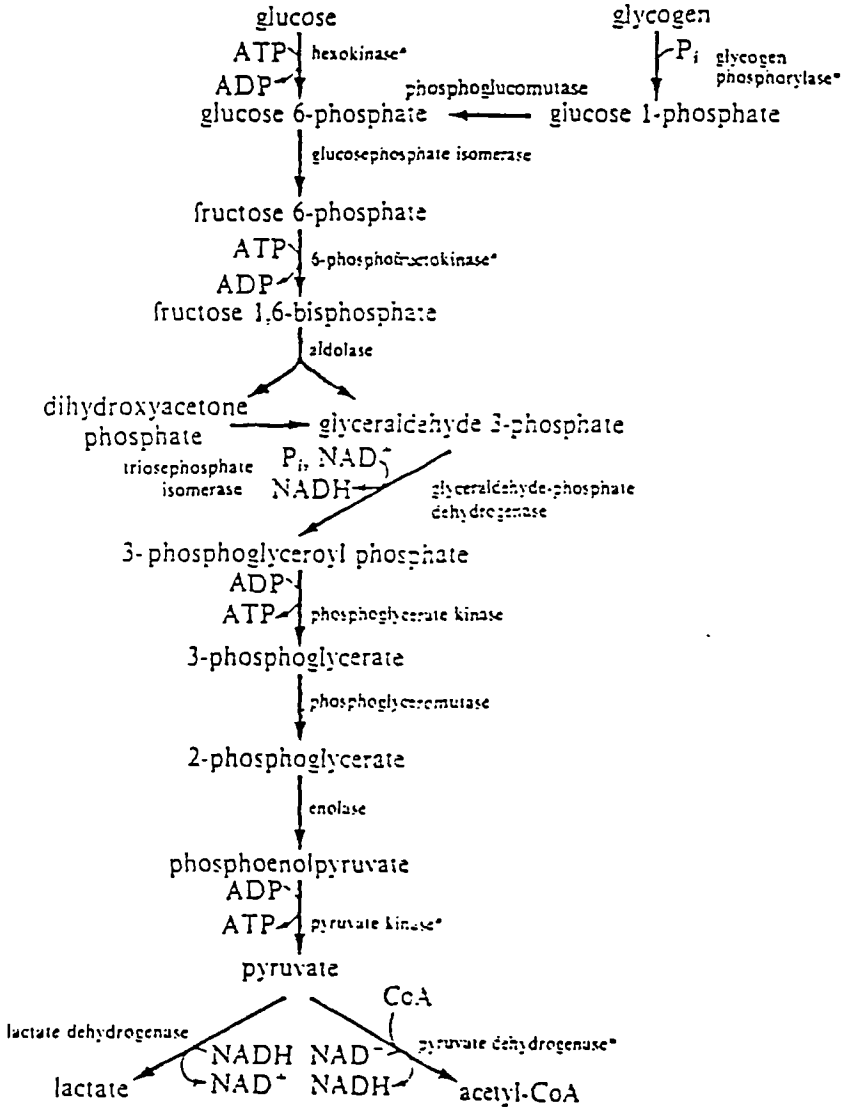

Figure 2.1. The glycolytic pathway (after Newsholme and Leech, 1983).

Lactate dehydrogenase is a near equilibrium enzyme in high concentrations and as such, it is sensitive to changes in substrate (i.e. pyruvate and extramitochondrial NADH) (Newsholme and Start, 1973). Under conditions of a high glycolytic flux, the enhanced rate of supply of both these substrates would thus favour a high rate of lactate production.

Until a few years ago, it was accepted that glycogenolysis leading to lactate formation contributed to energy demands of high-intensity exercise only after the intramuscular stores of ATP and PCr were exhausted or depleted to a critical level. Earlier studies using intermittent exercise protocols have demonstrated that this critical level is not obtainable within the first 10s of high-intensity exercise but these suggestions were based on blood lactate concentrations as reflective of anaerobic glycogen metabolism (Margaria, Oliva, di Prampero and Cerretelli, 1969; Edwards, Melcher, Hesser and Wigertz, 1971). This view, has also been supported in a study where biopsy samples were taken (Saltin and Essén, 1971). The importance of glycogenolysis during high-intensity exercise was perhaps first suggested in the work of Saltin and Karlsson (1971) who demonstrated that the initial rate of glycogen utilisation in exercising muscle increased exponentially as a function of exercise intensity (expressed relative to maximal oxygen consumption - VO_2 max). These authors reported utilisation rates of about 0.7mmol glucosyl units.$Kg^{-1}dm.s^{-1}$ at an intensity of 150% VO_2 max. It is now accepted that glycogenolytic processes occur almost immediately with the onset of maximal exercise. This view, is supported by the marked increases in muscle lactate concentration observed after 2-10s of maximal dynamic exercise (Boobis et al, 1982; Jacobs, Tesch, Bar-Or, Karlsson and Dotan, 1983; Jones et al, 1985), sustained isometric contractions at near maximum force (Bergström et al, 1971) or electrical stimulation (Hultman and Sjöholm, 1983b; Ren, Chasiotis, Bergström and Hultman, 1988). In these studies, the rate of glycogen breakdown is calculated to be of the order of 2.5-6.3mmol glucosyl units.$Kg^{-1}dm.s^{-1}$ (Table 2.4) contributing between 30 to 65% of the total anaerobic ATP turnover rate during the first 2-10s of high-intensity exercise (Table 2.1). It has been estimated that the maximum rate of anaerobic glycolysis during exercise is in the order of

1.5-2.2mmol glucosyl units.$Kg^{-1}dm.s^{-1}$ in the quadriceps femoris muscle of man, corresponding to a rate of ATP formation of 4.5-6.6mmol ATP.$Kg^{-1}dm.s^{-1}$ (Hultman et al, 1987), and the delay before glycolysis is maximally activated, is estimated to be 4-5 seconds (Bergström et al, 1971). It appears therefore, that glycogenolysis leading to lactate formation is probably a very significant source of energy during brief maximal exercise, along with PCr degradation. Furthermore, high-intensity continuous dynamic exercise or electrical stimulation lasting 30-60s has been reported to elicit changes in muscle glycogen at a rate of 2.2-3.4mmol glucosyl units.$Kg^{-1}dm.s^{-1}$ (Table 2.4) accounting for 60 to 90% of the total anaerobic ATP turnover rate (Table 2.1).

That anaerobic glycolysis makes a significant contribution to ATP resynthesis during high-intensity exercise is easily manifested by the considerable accumulation of muscle lactate observed during this type of exercise (Table 2.5). Rates of lactate production ranging from 2-5.3mmol.$Kg^{-1}dm.s^{-1}$ have been reported during maximal cycling or running lasting 30s (Boobis et al, 1982; Jacobs et al, 1983; Jones et al, 1985; Cheetham, Boobis, Brooks and Williams, 1986; McCartney et al, 1986; Nevill, Boobis, Brooks and Williams, 1989). In longer durations of dynamic or electrical stimulation exercise, rates of lactate production averaging 1mmol.$Kg^{-1}dm.s^{-1}$ have been reported (Costill, Barnett, Sharp, Fink and Katz, 1983; Hultman and Sjöholm, 1983a; Spriet, Söderlund, Bergström and Hultman, 1987a). These observations possibly suggest a reduced rate of glycogen degradation to lactate formation with time during intense exercise. Further support of this view, is the observation that total glycogen stores in skeletal muscle are reduced only by a small percentage (14-30% of resting value) following short term high-intensity exercise. In fact, if the high glyogenolytic rates reported during brief maximal exercise were continued, muscle glycogen stores would be depleted in less than 60s. It appears, therefore, that the rate of glycogen degradation to lactate formation is controlled during high-intensity exercise and glycogenolysis is unable to proceed to the point where glycogen stores are limited.

TABLE 2.5 Changes in muscle lactate ($mmol.kg^{-1}dm$) during high-intensity exercise.

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE LACTATE	POST-EXERCISE LACTATE
	CYCLING				
Hermansen & Vaage (1977)	Intermittent	3 x 60 (4min recovery)	9	4.8	115.1
Boobis et al (1982)	max	30	4M	9.3	28.4
		6		9.3	89.3
Jacobs et al (1982)	max	30	9F	9.0	60.5
Jacobs et al (1983b)	max	30	15M	6.0	73.9
		10		6.0	46.1
Jones et al (1985)	Isokinetic max (60rpm)	30	5M	9.4	162.6
		10	2M	9.4	74.8
	Isokinetic max (140rpm)	30	5M	10.5	152.1
		10	2M	10.5	84.7
McCartney et al (1986)	Isokinetic max (100rpm)	4 x 30 (4min recovery)	4M	6.1	124.3 (Sprint 1) 150.9 (Sprint 4)
Spriet et al (1989)	Isokinetic max (100rpm)	3 x 30 (4min recovery)	8M+F	62.1 67.8	95.4 (Sprint 2) 90.7 (Sprint 3)

TABLE 2.5 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE LACTATE	POST-EXERCISE LACTATE
	RUNNING				
Naveri et al (1978)	max	3 x 300m	5M	12.0	67.1
Costill et al (1983)	125% VO_2 max	82	6M	-	53.8 (gastrocnemius)
	400m	60	4M	-	84.7 (gastrocnemius)
Cheetham et al (1986)	max	30	8F	2.7	78.0
Nevill et al (1989)	max	30	16M+F	4.0	84.2
	VOLUNTARY ISOMETRIC				
Sahlin et al (1975)	68.3% MVC	to fatigue	8	3.1	106.5
Sahlin & Ren (1989)	Contraction 1	66-50% (MVC)	10M	3.4	95.0
	2 (2min after)		5M	67.0	100.0
	3 (4min after)		5M	43.0	100.0

TABLE 2.5 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	PRE-EXERCISE LACTATE	POST-EXERCISE LACTATE
	ELECTRICAL STIMULATION				
Hultman & Spriet (1986)	Intermittent (20Hz) (1.6s stimul-1.6s recovery)	80s	7M+F	5.3	71.8
Chasiotis et al (1987)	Intermittent 20Hz (25% MVC) (1.6s stimul-1.6s recovery)	10 22 54.4	4M+F	3.5	15.5 30.7 81.7
	Continuous 20Hz (25% MVC)	12 22 52		3.5	18.8 42.9 113.1
Ren et al (1988)	2 Contractions 20Hz (70% MVC) Recovery 60s-occlusion	10(1) 10(2)	10M	3.5 23.6	18.5 44.0
	2 Contractions 20Hz (70% MVC) Recovery 60s-intact circul.	10(1) 10(2)		2.8 13.7	19.1 29.7

Regulation of anaerobic glycogen degradation in skeletal muscle

The increase in muscle glycogen degradation during high-intensity exercise results from the interplay of at least three main regulatory mechanisms which control the activity of phosphorylase. In brief, these include; the transformation of phosphorylase from an inactive b form, at rest, to the active a form, mediated by Ca^{2+} increase in the cytoplasm or by a hormonal mechanism involving cyclic-AMP; the activation of phosphorylase by changes in the concentration of its substrates; and the allosteric activation of phosphorylase by exercise-induced alterations in metabolite levels.

When muscle contracts, Ca^{2+} released from the sarcoplasmic reticulum, induces glycogenolysis by activating phosphorylase b kinase and thereby stimulating the conversion of phosphorylase b to a form (Newsholme and Start, 1973). As the Ca^{2+} release in the cytoplasm initiates each muscle contraction, this would also mean that the frequency of contraction also determines the rate of glycogenolysis (Hultman, 1986). It has been reported however, that phosphorylase b to a conversion during dynamic exercise (100% VO_2 max) or sustained isometric contractions (66% MVC) or electrical stimulation (25% MVC) is transient and is rapidly reversed despite the maintenance of contractile activity and absence of muscle fatigue (Chasiotis, Sahlin, Hultman, 1982; Chasiotis, et al, 1987). This has also been demonstrated during continuous electrical stimulation in rat plantaris muscle (Conlee, McLane, Rennie, Winder and Holloszy, 1979). Possible mechanisms for that phenomenon include the effect of metabolite accumulation in muscle upon the kinetics of phosphorylase transformation (Chasiotis, Sahlin and Hultman, 1983a) or the decrease in muscle pH which favour reduction in phosphorylase b kinase activity (Danforth, 1965). However, when adrenaline was infused before and during isometric contractions only a partial blunting of the reversion of phosphorylase a to b form occurred (Chasiotis and Hultman, 1985), thus suggesting the need for adrenaline to maintain phosphorylase in its a form during muscular work.

The transformation of phosphorylase b to a form in the presence of adrenaline is mediated by cyclic-AMP activation of a protein kinase which transforms phosphorylase b kinase to active form and thus,

converts phosphorylase b to a (Newsholme and Start, 1973; Chasiotis, 1988). Few studies have examined cyclic-AMP levels in response to exercise in skeletal muscle. These studies have demonstrated increasing levels of cyclic-AMP in skeletal muscle by 1-3 fold of the resting values, during high-intensity dynamic exercise (Chasiotis et al, 1982) or electrical stimulation with adrenaline infusion (Chasiotis, 1988). In contrast, isometric contractions had little effect on cyclic-AMP levels (Chasiotis et al, 1982). It is possible that the type of exercise influences the cyclic-AMP response in skeletal muscle.

The importance of hormonal regulation per se in mediating muscle glycogenolysis during exercise has become the subject of controversy. Animal studies using b-adrenergic blockade have provided conflicting results during prolonged exercise (Juhlin-Dannfelt, Terblanche, Fell, Young and Holloszy, 1982; Issekutz, 1984). In contrast, b-blockade decreased the rate of glycogen degradation during high-intensity exercise (115% VO_2 max) in humans (Chasiotis, Brandt, Harris and Hultman, 1983). Moreover, some studies have reported increased glyogenolytic rates during prolonged electrical stimulation (30min) after adrenaline infusion (Spriet, Ren and Hultman, 1988) while others have shown no major changes during intense dynamic exercise (Chasiotis et al, 1982) or electrical stimulation (Chasiotis, 1988). Conclusively, although transformation of phosphorylase b to a form appears to be important in the presence of elevated levels of adrenaline, it is not always associated with high rates of glycogen breakdown in skeletal muscle. It would appear therefore, that the benefit of the rapid rise in circulating levels of plasma adrenaline (10-20 fold) that is observed during maximal dynamic exercise of short duration (MacDonald, Wootton, Munoz, Fentem and Williams; Naveri, Kuoppasalmi and Harkönen, 1985; Cheetham et al, 1986; Brooks, Nevill, Meleagros, Lakomy, Hall, Bloom and Williams, 1990) is to initiate the transformation of phosphorylase b to a form, in readiness for rapid mobilisation of glycogen (Chasiotis, 1988). Further support of this view, is the observation that intensity rather than duration of exercise is the primary determinant of the magnitude of the catecholamine response (Jezova, Vigas, Tatar, Kevtnansky, Nazar, Kaciuba-Uscilko and Kozlowski, 1985).

Although there are several reports in the literature that muscle glycogen degradation is related to its pre-exercise levels (Astrand and Rodahl, 1986), substrate regulation of glycogenolysis by Pi has been implicated as a more potential regulator during high-intensity exercise (Chasiotis et al, 1982). In support of this view, is the observation that low concentrations of free Pi at rest limited glycogenolysis, despite phosphorylase transformation to the a form, as occurred after adrenaline infusion (Chasiotis, Sahlin and Hultman, 1983b). However, at the onset of intense muscular contractions the decrease in PCr levels activated phosphorylase through the corresponding increase in Pi concentration and increased the rate of glycogen degradation (Chasiotis et al, 1982). Further support of this view, has recently been provided by inhibition of PCr resynthesis under ischaemic conditions between two bouts of electrical stimulation (Ren, Chasiotis, Bergström and Hultman, 1988). It was shown that this situation which resulted in increased Pi concentration and decreased PCr, activated glycogenolysis in the second bout. It was noted however, that no such activation occurred in the absence of muscular contraction during the resting interval (Ren et al, 1988). It appears therefore, that during high-intensity exercise the rapid increase in Pi concentration in muscle as a result of PCr degradation coupled with a high proportion of phosphorylase in the a form, possibly mediated by Ca^{2+} and adrenaline increase, provide a link between energy demand and glycogen utilisation. It has been suggested that an increased concentration of H^+ ions exerts a negative effect on glycogenolysis by decreasing the amount of Pi in the monoprotonated form (HPO_4^{2-}) which is believed to be the active substrate for phosphorylase (Chasiotis et al, 1983a). In frog sartorius muscle a decreased pH has also been shown to decrease the rate of phosphorylase b to a transformation through inhibition of phosphorylase b kinase during electrical stimulation (Danforth, 1965).

In addition to Pi, metabolite alterations associated with intense exercise may exert important allosteric effects in phosphorylase activity (Newsholme and Start, 1973). During high-intensity exercise, the concentrations of AMP[1] and IMP increase (Sahlin et al, 1978). These metabolites are known to be potent metabolic regulators in the activities of both phosphorylase b and a (Aragon, Tornheim and Lowenstein, 1980). Although the functional significance of increased AMP and IMP levels in the regulation of glycogenolysis is not clear, it has been suggested that they may be important in the maintenance of glycogen degradation by activating phosphorylase b particularly at a time when phosphorylase a activity is relatively low (Hargreaves and Richter, 1988). Others have suggested that the role of AMP serves as an amplification mechanism that can detect and magnify small changes in ATP concentration (Newsholme and Start, 1973).

During high-intensity exercise, several studies have shown considerable accumulation in a number of glycolytic intermediates. The rate and magnitude of these changes reflect both the total flux through the glycolytic pathway and the relative activity of the enzymes involved in its regulation. The flux through the reaction catalysed by phosphofructokinase (PFK) is usually considered to be rate-limiting for glycolysis. The activity of the enzyme is inhibited allosterically by high levels of ATP, which lower the affinity of the enzyme for fructose-6-phosphate (F-6-P) (Newsholme and Start, 1973). The activity of PFK is extremely sensitive to pH changes and the enzyme is almost completely inhibited at pH 6.4 in vitro (Danforth, 1965; Ui, 1966), the intracellular pH at exhaustion (Sahlin, 1978). During high-intensity exercise, this inhibition can partly be overcome by a decrease in ATP and a corresponding increase in ADP and AMP (Triverdi and Danforth, 1966) or IMP (Sahlin, 1978) and ammonia (Spriet, Söderlund, Bergström and Hultman, 1987b). At rapid rates of muscle stimulation, the increased levels of Pi, F-6-P and fructose-1,6-diphosphate (F-1,6-diP)

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1. The changes in AMP are considered to be transient and difficult to be seen in biopsy samples (Sahlin, 1986).

have also been shown to be potent activators of PFK (Newsholme and Start, 1973; Dobson, Yamamoto and Hochachka, 1986). In effect this means that the presence of positive modulators release PFK from ATP inhibition at low pH, such that, the physiological pH profile of PFK is extended beyond that reported to be inhibitory from in vitro studies. Furthermore, several activators acting simultaneously, allow greater flexibility in enzyme action with small changes in their concentrations.

Studies of energy metabolism during high-intensity exercise have shown a large increase in the concentrations of glucose-6-phosphate (G-6-P) and F-6-P about 10 to 15-fold above resting levels (Boobis et al, 1982; McCartney et al, 1986; Cheetham et al, 1986). This marked accumulation of hexose monophosphates has been attributed to the lower activity of PFK compared with that of phosphorylase and/or to inhibition of PFK activity as exercise continues. Hultman and Sjöholm (1983a) demonstrated that during the first 5s of electrical stimulation (75% MVC), the glycogenolytic rate was 2.5 times higher than the glycolytic. As exercise continued, the rate of glycogen degradation decreased but it always exceeded glycolysis, the ratio being 1.2 after 50s of stimulation. The very large accumulation of G-6-P that is observed at the early stages of high-intensity exercise, has been suggested to be responsible for the transformation of phosphorylase a to b by activating phosphorylase a phosphatase and inhibiting phosphorylase b kinase (Hultman, 1986). Furthermore, formation of hexose monophosphates is also important in regulating the rise in intracellular Pi concentration from the breakdown of phosphagens (ATP and PCr) and thus, lowering the availability of Pi for glycogenolysis (Chasiotis et al, 1983a).

Conclusively, the rate of glycogen breakdown during high-intensity exercise is controlled, possibly through a feedback inhibition mechanism, by the accumulation of glycolytic intermediates acting at the enzymatic levels of PFK and phosphorylase.

Oxidative contribution to ATP resynthesis during high-intensity exercise

At the onset of intense muscular activity, the store of oxygen (O_2) available to the muscle cells in the form of oxymyoglobin, dissolved oxygen and in the capillaries as oxyhaemoglobin is very small. In human quadriceps femoris muscle, this has been estimated to be about 1.9-2.2mmolO_2.Kg^{-1}dm or 2.2-2.6mlO_2.Kg^{-1}dm (Harris, Hultman, Kaijser and Nordesjö, 1975). This would be sufficient for the resynthesis of 12mmolATP.Kg^{-1}dm. Under appropriate conditions, it has been estimated that the local muscle oxygen stores may contribute up to 8-9% of the total anaerobic capacity as calculated from the maximal accumulated O_2-deficit (Hermansen and Medbö, 1984; Medbö, Mohn, Tabata, Bahr, Vaage, and Sejersted, 1988). Earlier studies, using intense intermittent exercise of brief duration (10-15s of work: 10-30s of rest) have shown that energy provision was predominantly derived from oxidative metabolism (Margaria et al, 1964; Saltin and Essén, 1971; Essén, Hagenfeldt and Kaijser, 1977) and that the oxymyoglobin store may provide up to 44% to the O_2-deficit, cycling at VO_2 max (Essén et al, 1977).

The maximal rate of ATP resynthesis from oxidative phosphorylation has been calculated to be around 2-2.7mmol ATP.Kg^{-1}dm.s^{-1} (Jorfeldt and Wahren, 1971; McGilvery, 1975; Blomstrand, Ekblom and Newsholme, 1986). However, during high-intensity exercise of short duration (i.e. <30s), the total oxygen delivery to the working muscles and consequently the rate at which ATP may be resynthesised, is relatively low because the adjustment in ventilation, cardiac output and oxygen transport are relatively slow processes. Even if delivery of oxygen to the muscle was maximal, it is unlikely that the aerobic contribution to total energy demand could exceed 30-35%, as ATP turnover rates as high as 10mmol ATP.Kg^{-1}dm.s^{-1} have been reported during maximal exercise (Boobis, 1987). Moreover, delays in the activation of mitochondrial respiration due to increases in ADP and Pi concentrations during such intensities would further limit the rate of ATP resynthesis by aerobic metabolism.

Several studies have estimated the relative contribution of oxidative metabolism to ATP resynthesis during high-intensity exercise, calculated from O_2-uptake and O_2-deficit measurements. These studies have

collectively shown a 20-40% contribution during intense uphill treadmill running (Hermansen and Medbo, 1984; Medbo et al, 1988) or high-intensity cycling (Serresse, Lortie, Bouchard and Boulay, 1988; Medbo and Tabata, 1989) lasting 15-30 seconds. That aerobic metabolism makes such a significant contribution to ATP resynthesis during this type of exercise is also evident by the large arterial-femoral venous oxygen difference ($169ml.l^{-1}$) that has been observed at the end of a 30s maximal isokinetic cycling test (Kowalchuk, 1985-cited in McCartney et al, 1986 p1168). In longer durations of high-intensity exercise lasting 30-90s, aerobic metabolism may contribute up to 50-60% of the total energy demand (Thomson and Garvie, 1981; Hermansen and Medbo, 1984; Medbo et al, 1988; Medbo and Tabata, 1989; Serresse et al, 1988). This enhanced contribution of aerobic metabolism with time during high-intensity exercise has been attributed to inhibition of glycogen degradation and substantial utilisation of fuel other than glycogen, possibly intramuscular triacylglycerol stores (McCartney et al, 1986). Indeed, the oxidation of free fatty acids would require an obligatory oxygen consumption. This suggestion however, was only supported by the large increases in plasma glycerol concentration (5-fold) observed in the above study although, considerable muscle lipolysis and decrease in human muscle triacylglycerol store content (20%) has been demonstrated by others within 5 minutes of intermittent exercise but at lower exercise intensities (100% VO_2 max) (Essén, 1978).

2.4 CHANGES IN ACID-BASE BALANCE DURING HIGH-INTENSITY EXERCISE

Determination of human skeletal muscle pH

During high-intensity exercise H^+ accumulate which result in pH changes within muscle and blood. It was not until 1972, that the pH of muscle biopsy specimens obtained at the end of exhaustive cycling exercise was determined for the first time (Hermansen and Osnes, 1972). In that study, it was shown that whilst the blood pH continued to decrease during intermittent exhaustive exercise, the pH of muscle homogenates reached to about the same level (6.4) after each exhaustive exercise bout. Because there appeared to be a finite level to which intramuscular pH could decrease before fatigue necessitated cessation of the exercise, it was concluded that the low muscle pH limited exercise performance. Since then, several biopsy studies have shown that the pH determined on muscle homogenates of human quadriceps femoris muscle lies at rest between 7.0-7.1 and during exhaustive exercise of short duration is decreased by about 0.5-0.6 units (Table 2.6). This corresponds to an increase in H^+ concentration from 100nmol.l^{-1} intracellular water at rest to 320-400nmol.l^{-1} intracellular water following exercise ($pH=-\log[H^+]$). In spite of the fact that muscle homogenate pH measurements do not reflect truly intracellular pH, because they represent an average pH for the entire tissue both intracellular and extracellular (see methods), most investigators refer to these pH values as either intracellular or intramuscular pH.

Recently, a new technique has been developed (^{31}P-NMR) for the study of high-energy phosphate metabolism in muscle during exercise. With this non-invasive technique, it has been found that the pH in resting human forearm muscles lies between 6.99-7.08 (Wilson, McCully, Mancini, Boden and Chance, 1988; Dawson, 1983) thus averaging to a value which agrees well with determination on muscle homogenates. However, lower intramuscular pH values have been reported in studies using ^{31}P-NMR during maximal voluntary contractions of human forearm flexor muscles (Wilson et al, 1988) than those noted in leg muscle homogenates obtained after exhaustive leg exercise (Sahlin, et al 1976; Sahlin, Alvestrand,

TABLE 2.6 Changes in pH of human quadriceps femoris muscle (unless specified otherwise) induced by dynamic exercise and isometric or electrical stimulation.

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE	METHOD EMPLOYED TO MEASURE pH	N	MUSCLE pH PRE-	POST-
	CYCLING					
Hermansen & Osnes (1972)	To exhaustion	100s	Homogenate	8	6.98	6.46
Sahlin et al (1976)	To exhaustion	6-12min	Homogenate	12	7.08	6.60
Sahlin et al (1978)	To exhaustion	10-11min	CO_2	8	7.00	6.40
King et al (1985)	Isokinetic max	30s	Homogenate	10	7.15	6.67
Sharp et al (1986)	Incremental $25Wmin^{-1}$ (90rpm)	To exhaustion	Homogenate	8	7.10	6.65
Bell & Wegner (1988)	max	60s	Homogenate	9	6.90	6.59
	RUNNING					
Costill et al (1983)	125% VO_2 max	82s	Homogenate	6	7.04	6.86
	125% VO_2 max	82s	Homogenate	6	7.03	6.88(g)
	400m	60s	Homogenate	4	7.03	6.33(g)
Nevill et al (1989)	max	30s	Homogenate	16	7.02	6.80
Allsop et al (1990)	max	30s	pH-electrode	10	7.17	6.57

Note: (g) refers to gastrocnemius, (a) refers to adductor pollicis and (f) refers to forearm muscle.

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE	METHOD EMPLOYED TO MEASURE pH	N	MUSCLE pH PRE-	MUSCLE pH POST-
	VOLUNTARY ISOMETRIC					
Sahlin & Ren (1975)	68.3% MVC	45s	Homogenate	10	7.09	6.56
Sahlin & Henriksson (1984)	61.0% MVC	50.6s	Homogenate	8	7.12	6.61 (untrained)
	61.0% MVC	57.2s	Homogenate	7	7.10	6.80 (trained)
Miller et al (1988)	Sustained 75% MVC	4min	^{31}PNMR	-	7.08	6.58(a)
	Intermittent 75% MVC	5min	^{31}PNMR	-	7.08	6.55(a)
Wilson et al (1988)	Intermittent max 1s contractions	4min	^{31}PNMR	17	7.01	6.24(f)
	ELECTRICAL STIMULATION					
Spriet et al (1987b)	Intermittent 20Hz (1.6s stimul-1.6s)	25.6s	Homogenate	7	7.00	6.70
		51.2s				6.60
		76.8s				6.45
		102.4s				6.43

Brandt and Hultman, 1978). In these ^{31}P-NMR studies intramuscular pH has been reported to decrease by approximately a full pH unit to about 6.1-6.2 after contraction to fatigue (Wilson et al, 1988). Although the reason for the discrepancy between these two techniques is at present not known it has been argued that ^{31}P-NMR measures truly intracellular pH (Wilson et al, 1988; Miller, Boska, Moussari, Carson and Weiner, 1988). This is probable, because during high-intensity exercise extracellular pH is known to be considerably higher than intracellular pH (Sahlin et al, 1978) and therefore, pH determinations of muscle homogenates should be expected to produce a higher pH value than does ^{31}PNMR. Indeed, intracellular pH on muscle biopsy samples determined by the distribution of CO_2 in muscle, have been estimated to be 6.04 at the end of exhaustive cycling exercise compared to a pH 6.64 determined on muscle homogenate, although this low value was attributed to experimental error (Sahlin et al, 1978).

As a consequence of intense glycolytic activity during short-term intense exercise, most of the produced H^+ (94%) within the muscle has been related to the accumulation of lactic acid, with the remainder being provided by accumulation of other acids (i.e. pyruvate 0.3%; malate 3%), G-6-P (2%) and glycerol-1-phosphate (1%), (Hultman and Sahlin, 1980). If these H^+ were added to an unbuffered solution, the concentration of H^+ would be 35mmol.l^{-1} intracellular water and pH would decrease to about 1.5 (Hultman and Sahlin, 1980). As muscle pH decreases to approximately 6.5 or 6.4 following short-term exhaustive exercise, it appears that most of the released H^+ is buffered within the tissue and only a small fraction appears as free ions (less than 0.001%), (Sahlin, 1986). Thus, the magnitude of the decrease in muscle pH is determined both by the degree of lactic acid accumulation and by the ability of the muscle to buffer hydrogen ions.

Determinants of buffering capacity of human skeletal muscle and blood

During high-intensity exercise H^+ are released within the muscle. In considering muscle metabolism and function under such conditions, changes in acidity have important effects on the cellular biochemistry because both the rate of enzyme-catalysed reactions and the contractile mechanism are usually strongly affected (see section 2.7). The extent

of muscle pH decrease after addition of H^+ is determined by the buffering capacity[1]. There are several factors that determine the buffering capacity of the muscle. These have been broadly divided into physicochemical buffering, consumption or production of non-volatile acids and transmembrane fluxes of H^+ or HCO_3^- (Siesjo and Messeter, 1971; cited in Hultman and Sahlin, 1980 p69).

Physicochemical buffering occurs in the muscle cell merely as a consequence of the uptake of H^+ by weak bases present in the muscle and is independent upon the nature of the pH change (at a constant PCO_2). The most important contributors to physicochemical buffering are phosphate compounds, muscle proteins and the carbon dioxide/bicarbonate (CO_2/HCO_3^-) system. The muscle cell has a high concentration of phosphate compounds such as ATP, ADP PCr and Pi with pka values[2] within the physiological range. However, it is believed that a major fraction of ATP (pka = 6.9) content (approximately 90%) is complex-bound to Mg^{2+} in the muscle cell (Dawson, Gadian and Wilkie, 1978; Sahlin, 1978). Similarly, a major fraction of ADP (pka = 6.7) content (approximately 90%) is thought to be protein-bound (McGilvery, 1975). Moreover, due to the low pka value of the PCr (pka = 4.5) its contribution to cellular buffering by physicochemical processes would seem to be negligible despite its high concentration. Thus free Pi, is considered to be the most effective buffer from all phosphate compounds because it has a pka

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1. The buffering capacity of a solution expresses the resistance of that solution to pH changes when a strong acid or base is added and it has been defined by Van Slyke (1922) as the amount of free H^+ or OH^- required to produce a change of one pH unit. The unit for buffering capacity in $mmol.pH^{-1}.l^{-1}$ muscle water is designated as Slyke (Sl), (Woodbury, 1965).
2. The pka of an acid is the pH at which it is half-ionised. Buffers work best for pH values near their pk and the change in pH is inversely proportional to the total concentration of buffer in solution, i.e. concentrated buffer can operate effectively over a wider pH range compared to a diluted buffer.
   pka = -logka where ka is the acid constant.

value of 6.8 and a high concentration (38.6mmol.Kg^{-1}dm) in human skeletal muscle (Sahlin et al, 1978).

The content of protein in muscle tissue is high and its contribution to physicochemical buffering is important because certain of the aminoacids in the protein molecule, function as bases that bind readily with excess H^+. It has been estimated that proteins may contribute up to 40% of the total physicochemical buffering in human quadriceps femoris muscle during isometric contraction to fatigue (Hultman and Sahlin, 1980). The contribution made by free aminoacids to buffer capacity in the physiological range is limited to those demonstrating a pka for their ionizable groups in that range. As all free a-aminoacids have a pka <3 for the a-carboxyl group and a pka >9 for the a-amino group, their buffering power is thus dependent on the acid-base behaviour of the R-group, which however, changes when are incorporated into peptides and proteins. For instance, the pka of the imidazole-group belonging to histidine increases from 6.0 to 6.8 when incorporated into the dipeptide carnosine (Hultman and Sahlin, 1980). Protein-bound histidine and the imidazole -containing compound carnosine are considered to be major buffering components within human skeletal muscle (Parkhouse and McKenzie, 1984). The concentration of histidine in human skeletal muscle proteins has been determined to be of an order of 152mmol.Kg^{-1}dm (2.7g/100g protein) (Furst, Jansson, Josephson and Vinnars, 1970) and that of carnosine about 14-22.5mmol.Kg^{-1}dm (Parkhouse, McKenzie, Hochachka, Mommsen, Ovalle, Shinn and Rhodes, 1983). It has been calculated that over a muscle pH change from 7.0 to 6.6 the stoichiometric uptake of H^+ would be approximately 48mmol.Kg^{-1}dm (Hultman and Sahlin, 1980).

The CO_2/HCO_3^- system also contributes to physicochemical buffering. Direct measurements in human skeletal muscle have shown that intracellular HCO_3^- is approximately 9-10mmol.l^{-1} muscle water at rest and falls to 3mmol.l^{-1} after exhaustive cycling exercise lasting 10-11 minutes (Sahlin, 1978) or after 30s maximal isokinetic cycling (Kowalchuck, Heigenhauser, Lindinger, Sutton and Jones, 1988). It has been estimated that bicarbonate could contribute as much as 15-18% to total buffer capacity (approximately 12 Slykes) in vivo, during dynamic

exhaustive exercise, when intrumuscular pH decreases from 7.0 to 6.4 (Sahlin, 1978). With intact muscle circulation buffering capacity due to CO_2/HCO_3^- system, at pH = 7.0 and PCO_2 = 5.2kPa, amounts to 23 Slykes (Hultman and Sahlin, 1980).

In addition to physicochemical buffering which is only dependent upon the amount of weak bases and their dissociation constants, consumption or production of non-volatile acids is also important to total muscle buffering. This comprises of buffering which occurs by metabolic processes as a consequence of enzymatic activity during exercise. For example, utilisation of PCr (pka = 4.5) causes absorption of H^+ but the stoichiometry of the creatine-kinase reaction depends upon pH because of the direct involvement of H^+ in the equilibrium of that reaction. It has been estimated that at an intracellular pH of 7.08 for each molecule of PCr broken down, 0.34 mole of H^+ is taken up, whereas at pH of 6.6 the uptake of H^+ is 0.61 mole (Hultman and Sahlin, 1980). However, under intracellular conditions several other factors could affect the stoichiometry of the creatine-kinase reaction, such as the free concentration of Mg^{2+}, pkaATP, pkaADP and the dissociation constants of the Mg^{2+} and K^+-complexes of the nucleotides (Sahlin et al, 1975). It appears therefore, that calculation of metabolic buffering processes are only valid under the prevailing conditions. Deamination of AMP during exercise via the purine nucleotide pathway also contributes to metabolic buffering. As muscle ATP and TAN content decreases, IMP concentration increases in a stoichiometric fashion (Sahlin et al, 1978; Spriet et al, 1987a) but the stoichiometrical uptake of H^+ is estimated to be only 0.41 mole per mole IMP formed. The total amount of H^+ taken up by this reaction during high-intensity exercise is rather small due to the relatively small amount of IMP formed. The increase in IMP content is accompanied by an equimolar accumulation of NH_4^+ as significant reamination does not occur. Ammonia production may also act as a buffer of hydrogen ions, but is of minor importance (Katz, Sahlin and Henriksson, 1986a). It should be noted, that the metabolic processes involved in the H^+ balance and therefore regulation of muscle pH are dependent upon the overall metabolism because their main task is not to maintain a constant pH but to generate the required energy during exercise.

The transmembrane fluxes of H^+ and HCO_3^- also affect the H^+ balance of the muscle cell and are determined by the permeability through the cell membrane, by the ionic composition of the extra- and intracellular fluids and by the membrane potential. These factors are discussed in Section 2.5. A linear relationship has been found between the muscle content of lactate and pyruvate and the decrease in pH of muscle homogenates taken immediately either after isometric contraction at 68% MVC (Sahlin et al, 1975) or after short-term (6-12min) exhaustive cycling exercise (Sahlin, Harris, Nylind and Hultman, 1976). It appears from these relationships that for a given concentration of muscle lactate, the decrease in muscle pH is more pronounced after isometric contractions than after dynamic exercise. With a closed circulation, when the local blood supply is occluded, muscle buffering is limited to physicochemical and metabolic processes, as transmembrane flux of H^+ and other ions is essentially inhibited. In addition, physicochemical buffering is restricted to Pi and protein compounds, as the CO_2/HCO_3^- system does not function in a closed system.

With intact circulation H^+ enters into the circulation where it causes changes in the acid-base status of the blood. The buffering processes of the blood are very important in order to maintain the concentration gradient of H^+ from muscle to the circulation. The uptake of H^+ from the circulation is provided primarily by haemoglobin, plasma proteins and the plasma bicarbonate system ($H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$). Immidazole groups are responsible for much of the buffering carried out by haemoglobin (Hb) and other proteins of the blood (pka range 6-7). Although there is a relatively low concentration of Hb in the blood ($2.33 mmol.l^{-1}$), it functions as a more effective buffer than, say, phosphate because it has more buffering groups per molecule. The equilibrium of the CO_2/HCO_3^- reaction is determined by the concentrations of the molecules and ions involved. If CO_2 can be removed from the system, the reaction is maintained to the right. In normal conditions, the respiratory system in the brain regulates pulmonary ventilation so that the partial pressure of CO_2 (PCO_2) in arterial blood is kept at 5.3kPa (40mm Hg). The buffering capacity of the bicarbonate system at PCO_2=5.3kPa and pH=7.4 is 55 Slykes in plasma (Hultman and Sahlin, 1980). Any increase in the quantity of H^+ in extracellular fluids and

plasma as a result of exercise, increases CO_2-tension which in turn stimulates the respiratory centre and causes an immediate increase in ventilation. This adjustment rapidly reduces alveolar PCO_2 and causes CO_2 to be removed from the blood. In effect, the reduction in plasma CO_2 facilitates the recombining of H^+ and HCO_3^-, thus lowering the plasma bicarbonate fraction of the blood. Resting plasma bicarbonate concentration has been estimated to be of an order of 25-26mmol.l^{-1} (Sharp, Armstrong, King and Costill, 1983) and may decrease to 5mmol.l^{-1} after performing high-intensity exercise of short duration (Osnes and Hermansen, 1972).

Several methods have been employed to determine the buffering capacity of skeletal muscle. This has been estimated by titration of muscle homogenates with strong acid or base or CO_2, by theoretical calculations based upon the physicochemical constituents of muscle cell contents; and, by calculation from the estimated release of H^+ (i.e. lactate accumulation) and the observed change in muscle pH (known as apparent buffer capacity). Values for the buffering capacity of human skeletal muscle are shown in Table 2.7.

TABLE 2.7 Buffering capacity ($mmolH^{+}.l^{-1}$ muscle water pH^{-1}) of human quadriceps femoris muscle (unless specified otherwise)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE	METHOD EMPLOYED	N	MUSCLE BUFFERING CAPACITY
Sahlin et al (1976)	To exhaustion	6-11min	$\frac{\Delta La^{*}}{\Delta pH}$	12	73
Sahlin et al (1978)	To exhaustion	10-11min	$\frac{\Delta La^{*}}{\Delta pH}$	8	77
Sharp et al (1986)	Incremental $25Wmin^{-1}$ (90rpm)	To exhaustion	$\frac{\Delta La^{*}}{\Delta pH}$	8	58.1 (pre-training) 79.4 (post-training)
Bell & Wenger (1988)	max	60s	HCl-titration	9	64.8 (pre-training) 75.1 (post-training)
	RUNNING				
Parkhouse et al (1983)	$3m.s^{-1}$ 20% incline	To exhaustion	HCl-titration (deproteinised)	5 5 4	27.1 (endurance) 39.1 (sprinters) 29.1 (untrained)
Costill et al (1983)	125% VO_2 max	82s	$\frac{\Delta La^{**}}{\Delta pH}$	6 6	107.0(g) 78.0
	400m	60s	$\frac{\Delta La^{**}}{\Delta pH}$	4	64.0(g)
Nevill et al (1989)	max	30s	HCl-titration	16	65.7

Note: *muscle water assumed 77%, **calculated by the present author, (g) refers to gastrocnemius

TABLE 2.7 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE	METHOD EMPLOYED	N	MUSCLE BUFFERING CAPACITY
	VOLUNTARY ISOMETRIC				
Sahlin et al (1975)	68.3% MVC Occlusion	MVC>40%	$\frac{\Delta La^*}{\Delta pH}$	10	57.0
Sahlin & Henriksson	61.0% MVC Occlusion	To exhaustion	$\frac{\Delta La^*}{\Delta pH}$	8 7	49.7 (untrained) 58.6 (trained)
	ELECTRICAL STIMULATION				
Spriet et al (1987c)	Intermittent 20Hz (1.6s stimul-1.6s recovery)	102.4s	$\frac{\Delta La^*}{\Delta pH}$	7	77.5

2.5 MUSCLE METABOLISM DURING RECOVERY FROM HIGH-INTENSITY EXERCISE

Resynthesis of phosphorylcreatine

During recovery from high-intensity exercise, it was originally thought that total PCr resynthesis continued along a single exponential curve; the half time of resynthesis (i.e. the alactacid fraction of the oxygen-debt repayment) estimated to be about 25-30s (Margaria et al, 1969). It was later shown, that the time-course of PCr resynthesis is biphasic exhibiting both a fast and a slow component (Harris, Edwards, Hultman, Nordesjö, Nylind and Sahlin, 1976). The half-time for the fast component was 21-22.5s whereas the half-time of the slow component was more than 170s following either dynamic or isometric exhaustive exercise.

During recovery from intense exercise under aerobic conditions, the relationship between PCr and lactate disappears (Harris et al, 1977). Phosphorylcreatine is rapidly resynthesised while muscle lactate remains high. The rate of PCr resynthesis within the muscle cell, is thought to be regulated primarily by the availability of ATP from oxidative metabolism. The dependence of rapid ATP resynthesis on oxygen has been supported by the finding that PCr resynthesis during recovery from exercise is completely abolished when the circulation to the quadriceps femoris is occluded and partial restoration of the PCr content occurred rapidly only after release of occlusion, while muscle lactate remained high (Harris et al, 1976). Thus, it appears that the immediate effect of the restored circulation was to increase the availability of oxygen in the muscle cell rather than to change cellular pH by transport of H^+ outside the cell (Harris et al, 1976). The dependence of PCr resynthesis on oxygen availability has also been confirmed by incubation of tissue samples in atmospheres of oxygen and nitrogen (Sahlin, Harris and Hultman, 1979). Fifteen minutes of incubation in oxygen increased the PCr content from 4% to 68% of the value at rest inspite of unchanged pH, but incubation in nitrogen left the PCr content unchanged (Sahlin et al, 1979).

It is also possible that the rate of PCr resynthesis after intense exercise is limited by the recovery in muscle pH, since a low intracellular pH would shift the equilibrium of the creatine-kinase reaction in favour of PCr degradation. The slow-component of PCr resynthesis may, partly be due to the slow recovery of muscle pH after high-intensity exercise (Harris et al, 1976; Naveri, Rehunen, Kuoppasalmi, Tulikoura and Harkonen, 1978). Furthermore, as most of the Pi bound up in the formation of organic phosphates during exercise would have been derived partly from the breakdown of PCr, it is reasonable to suggest that PCr resynthesis during recovery is also linked to the decay of these phosphates.

Following exercise with full circulation, the initial rate of PCr resynthesis has been calculated to be 2-3mmol.Kg^{-1}dm.s^{-1} (Harris et al, 1976). However, other reports have indicated much slower rates of resynthesis (0.4-1.7mmol.Kg^{-1}dm.s^{-1}) for the first 60s of recovery (Essén and Kaijser, 1978; Hultman, Sjöholm, Sahlin and Edström, 1981; Ren et al, 1988). This suggests that the conditions of the preceding exercise itself might be of importance to the rate of resynthesis.

Efflux of H^+ and lactate from skeletal muscle during exercise and recovery - restoration of normal acid-base balance

Protons associated with lactate formation accumulate within the muscle during high-intensity exercise and are released to the blood (Table 2.8) and extracellular fluids, eventually restoring muscle acid-base balance to normal levels during recovery. In active muscle, it seems likely that a large amount of acid efflux is associated with the movement of undissociated acid produced by metabolism. This is certainly true for CO_2 which is present in high concentrations and passes readily through cell membranes. It has been a matter of debate, however, whether lactic acid permeates through the muscle cell membrane in ionic form as lactate anion or as an undissociated acid molecule. It has been demonstrated, in vitro, that passive diffusion of lactic acid could occur through the cell membrane at relatively high external pH and high buffer concentration and that the rate of diffusion was dependent upon extracellular pH (Mainwood and Worsley-Brown, 1975). At physiological pH values, the efflux of undissociated lactic acid appears to be less

TABLE 2.8 Changes in blood pH and lactate ($mmol.l^{-1}$) induced by dynamic exercise

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	BLOOD pH Pre-	BLOOD pH Post-	LACTATE Post-
	CYCLING					
Hermansen & Osnes (1972)	To exhaustion	100	5	7.40	7.18	- (c)
Osnes & Hermansen (1972)	Intermittent	5 x 40-60 (4min recovery)	14	7.43	6.80	32.1 (c)
Keul & Doll (1973)	Intermittent	6 x 120	10	-	-	13.2 (v)
Wootton & Williams (1983)	Intermittent	5 x 6 (30s recovery)	16	-	-	15.5 (c)
McCartney et al (1986)	Intermittent 100 rpm Isokinetic max	4 x 30 (4min recovery)	8	-	7.10	23.0 (v)
Kowalchuck et al (1988)	Isokinetic max 100rpm	30	3	-	7.02	18.0 (v)
	RUNNING					
Cheetham et al (1986)	max	30	8	7.40	7.16	13.1 (c)
Hirvonen et al (1987)	max	11 (100m)	7	7.42	7.24	8.2 (c)
Medbo & Sejersted (1985)	6^{o} incline	To exhaustion (60s)	6(Sprinters) 6(Endurance)	7.41 7.43	7.07 7.19	16.7 (a) 12.3 (a)
Medbo et al (1988)	6^{o} incline To exhaustion	15 (250% VO_2 max) 30 (180% VO_2 max) 60 (150% VO_2 max)	11 11 11	- - -	- - -	9.4 (c) 12.5 (c) 14.8 (c)

TABLE 2.8 (continued)

REFERENCES	TYPE OF EXERCISE	DURATION OF EXERCISE (s)	N	BLOOD pH Pre-	BLOOD pH Post-	LACTATE Post-
Brooks et al (1988)	max	30	10	7.38	7.16	13.5 (v)
Brooks et al (1990)	Intermittent max	10 x 6 (30s recovery)	9	7.38	7.05	14.0 (v)

Note: (c) refers to capillary, (v) refers to venous and (a) refers to arterial blood

probable since, the lactic acid formed in the muscle cells is almost completely dissociated (pk = 3.7; pH = 7.0; lactate/H^+lactate$^-$ = 2000) and only 0.05% of total intracellular lactate is in a non-ionised form (Hultman and Sahlin, 1980). Thus, it has generally been assumed that the lactate anion is the permeating species, although a limited rate of lactate efflux in association with protons does occur. This efflux reaches levels of the order of 400nmol.g^{-1} wet muscle.min^{-1} in frog sartorius muscle (Mainwood and Worsley-Brown, 1975).

The exact mechanisms regulating the efflux of H^+ and lactate in ionic form out of the muscle have not been fully elucidated and may involve passive diffusion, ionic exchanges and/or an active transport mechanism (Hultman and Sahlin, 1980). Both H^+ and lactate ions are charged and therefore the driving force for passive diffusion of these ions over the cell membrane should be determined by the difference in electrochemical potential between muscle and blood. It has been suggested that during exhaustive dynamic exercise the difference is reduced and in exhausted muscle the membrane potential is probably decreased below the value required to maintain H^+ in electrochemical equilibrium over the cell membrane, thus, making a passive efflux of H^+ possible (Sahlin, 1978). Moreover, the negative intracellular interior would exert an electrical force to drive the negatively charged lactate ions out of the cell. Manipulation of extracellular lactate concentration by lactate infusion has also been shown to influence the rate of net lactate release in the circulation during forearm exercise (Jorfeldt, 1970).

Two types of ionic exchange mechanisms which conceivably influence the outward transport of H^+ and lactate ions have been identified in resting mouse soleus muscle. These are the Na^+/H^+ exchange and anion exchange transport systems (Aickin and Thomas, 1977). Involvement of the Na^+/H^+ exchange system in the regulation of intracellular pH during recovery following stimulation of mouse soleus muscle has been demonstrated recently (Juel, 1988), although it does not appear to be of primary importance in amphibian muscle (Allard, 1988). In addition, Juel (1988) reported that intracellular pH recovery was clearly dependent on the buffer concentration of the medium, thus confirming previous observation in frog muscle (Mainwood and Worsley-Brown, 1975), but independent of

the type of buffer. This observation, seems to exclude the possibility that bicarbonate efflux is involved in muscle pH recovery via a lactate/HCO_3^- exchange or HCO_3^- /Cl^- exchange (Hirche, Hauback, Langohr, Wacker and Busse, 1975).

It has been shown in vitro, that intracellular pH recovers more rapidly than lactate efflux in stimulated rat diaphragm and frog sartorius muscle; the rate of H^+ efflux exceeding that of lactate ions by factors of about 14 and 50, respectively (Benade and Heisler, 1978). This dislocation of H^+ and lactate efflux has also been demonstrated in human muscle. Sahlin and co-workers (1976) reported that during the first minute of recovery from exhaustive cycling exercise no further decrease in muscle pH occurred despite a calculated production of 34mmol $H^+.Kg^{-1}$dm that should have been occurred due to PCr resynthesis. In addition, in that study a linear relationship was observed between muscle lactate and pyruvate content and muscle pH for the whole recovery, indicating that the efflux of H^+ was faster than for lactate ions during the early part of the recovery period.

With the exception during the early phase of recovery, lactate and H^+ ions pass out of the muscle cell at approximately the same rate. It has been demonstrated in vitro that most of the lactate is removed by a lactate/H^+ cotransport mechanism (Allard, 1988). Several studies have further shown that total acid load exceeded the lactate accumulation in blood in the immediately post-exercise period (Osnes and Hermansen, 1972; Sahlin et al, 1978; Medbo and Sejersted, 1985). This was indicated by the higher increase in blood base-deficit than could be accounted for by lactate accumulation in blood, provided the distribution volume of the two ions is equal. That muscle release of H^+ exceeds the release of lactate would also explain the rapid initial fall in blood pH and slower increase in blood lactate often observed after maximal exercise (Medbo and Sejersted, 1985; Cheetham et al, 1986). The identification of a maximal rate of lactate release may account for these findings (Jorfeldt, Juhlin-Dannfelt and Karlsson, 1978). It was found that the release of lactate increased linearly with the muscle lactate content during submaximal cycling exercise to about 4-5mmol.min^{-1} per leg, occurring at a muscle content of about

17mmol.Kg^{-1}dm. A further increase of muscle lactate did not increase the efflux rate indicating possible saturation of the lactate translocation process. These lactate translocation hindrances were attributed to factors located in the cell membrane (Jorfeldt et al, 1978). It is also possible that the increased water content of muscle during exhaustive exercise (Bergström et al, 1971) also restricts lactate diffusion into the circulation. Recently, however, it has been reported that one-third of the lactate produced during one-leg intense cycling to exhaustion, was released to the blood during the exercise and the rate of release was as high as 27mmol.min^{-1} for one subject (Bangsbo, Gollnick, Graham, Juel, Kiens, Mizuno and Saltin, 1990). It has been shown in vitro, that the lactate transport mechanism is not saturated under physiological conditions and that lactate efflux is linearly related to intracellular concentration (Juel, 1988).

The recovery in muscle pH after short-lasting cycling to exhaustion (6-12min) follows an exponential time course with a half-time of about 10 minutes. After a 20 minute passive recovery, muscle pH is restored to the pre-exercise value (Sahlin et al, 1976). If the local blood supply to the muscle is inhibited, no recovery occurs in muscle pH after termination of exercise (Sahlin et al, 1975). This stresses the importance of the circulatory system for the recovery processes in muscle. In a recent study, using a needle tipped pH-electrode a plateau in muscle pH was observed after 2 minutes of recovery, following maximal running, with no further improvement until after 10 minutes of recovery (Allsop, Cheetham, Brooks, Hall and Williams, 1990). It was suggested that this plateau was possibly a purely extracellular phenomenon, thus reflecting a continuing efflux of H^+ from within the muscle cell after cessation of exercise. Recovery of muscle pH has also been measured by ^{31}PNMR after isometric contraction and seemed to follow the course of lactate except that a further decrease in muscle pH occurred during the first minute of recovery (Taylor, Bore, Styles, Gadian and Radda, 1983). The post contraction decrease in muscle pH was suggested to be due to the fast resynthesis of PCr, which produced H^+. Finally, the attainment of resting muscle pH following intense exercise does not appear to be associated with complete restoration of the buffering capacity of the

muscle. It has been shown, that intramuscular bicarbonate levels were still markedly reduced 20 minutes after performing exhaustive exercise (7.08 vs 10.2mmol.l^{-1} at rest) (Sahlin et al, 1978).

The efflux of protons into the circulation, causes changes in the acid-base status of the blood. The uptake of H^+ from the circulation is provided primarily by haemoglobin, plasma proteins and the CO_2/HCO_3^- system. Finally, the excretion of H^+ ions by the kidney, is of utmost importance if the alkali reserve of the body is to be maintained.

Metabolic fate of muscle lactate during recovery

The decay curve for muscle lactate in humans following exhaustive cycling exercise has been shown to proceed exponentially with respect to time (half-time approximately 10min) at an initial rate of 7.7mmol.Kg^{-1}dm.min^{-1} (Sahlin et al, 1976). During the early phase of recovery (1-5min), following high-intensity exercise, several studies have shown that blood lactate concentration increases (Cheetham et al, 1986; Rieu, Duvallet, Scharapan, Thieulart and Ferry, 1988; Brooks, Burrin, Cheetham, Hall, Yeo and Williams, 1988) thus, suggesting that during that period the rate of lactate transport into the blood is higher than the rate of lactate removal. Blood lactate is removed from the circulation at rest during recovery, by the liver, kidney, heart, brain and skeletal muscle and its ultimate fate is oxidation (via the citric acid cycle) or glyconeogenesis (via the Cori cycle).

It was originally thought that the liver was the primary post-exercise fate of lactate and practically all lactate taken up was utilised for glucose production (85%) with the remaining oxidised to carbon-dioxide and water (Krebs, 1964). Recently, it has been shown that lactate uptake by the liver accounted for only 10% of the total lactate formed during combined arm and leg exhaustive cycling exercise (Astrand, Hultman, Juhlin-Dannfelt and Reynolds, 1986). This was attributed to low hepatic post-exercise venous pH. Indeed, it has been demonstrated that lactate uptake and gluconeogenesis in rat liver were inhibited by acidosis (Iles, Cohen, Rist and Baron, 1977). It appears, therefore,

that the contribution made by the liver to lactate removal during recovery following high-intensity exercise is relatively small despite hepatic restoration of blood supply.

Although studies of blood lactate kinetics by isotope tracer techniques do not provide direct information about the sites of lactate removal, skeletal muscle is being heavily implicated in this process. Data from studies of intact rats (Brooks, Brauner and Gassens, 1973) and humans (Jorfeldt, 1970) have indicated that during sustained exercise intensities (40-75% VO_2 max), most of the lactate formed was removed by oxidation (70-90%) within the active muscle tissue either directly or upon reperfusion. The issue of lactate removal during high-intensity exercise, is unfortunately little helped by isotope tracer techniques for they require the presence of a steady or near steady state. In a more recent study it was suggested that oxidation was also the primary post-exercise fate of lactate, although some direct conversion of lactate to glycogen and aminoacids (alanine cycle) was not ruled out (Brooks and Gaesser, 1980). It should be noted however, that the supporting data in that study were obtained from rats exhausted by prolonged exercise and hence may not represent lactate removal following high-intensity exercise that results in large lactate accumulation, considerable decrements in muscle and blood pH and small reduction of glycogen stores. Indeed, it has been shown that the major fraction (75%) of lactate formed during high-intensity exercise was converted directly to glycogen (the content of which increased by approximately 2.4mmol glucosyl units.Kg^{-1}dm.min^{-1}) via glyconeogenesis during the 30min recovery period and only a small fraction of the lactate removal (15%) could be accounted for by oxidation or utilisation via the alanine cycle (Hermansen and Vaage, 1977). However, the results of that study have been questioned for lack of consideration of the distribution volume for lactate in the total body and consequently the calculations of lactate to glycogen conversions pertained only to the leg muscles (Brooks and Gaesser, 1980). Recently, assuming a distribution volume for lactate of 55% of the body mass and accounting for splachnic metabolism, it has been calculated that approximately 50% of the lactate formed during intense exhaustive exercise using both arm and leg muscles was transformed to glycogen in muscle during the first hour of recovery

(Astrand et al, 1986). It has also been demonstrated, using [^{14}C] lactate, that fast-twitch plantaris muscle of rats has the capacity for synthesizing glycogen from lactate at physiological significant rates, but slow-twitch soleus muscle does not, due to an extremely low level of fructose-1,6-diphosphatase activity (McLanne and Holloszy, 1979). It appears therefore, that the biochemical apparatus for glyconeogenesis is available at least in fast-twitch muscle. The possibility that lactate generated during high-intensity exercise may serve, at least partially, as a precursor for muscle glycogen resynthesis has also been raised in a study which showed considerable resynthesis of muscle glycogen in the absence of food intake within two hours following repeated bouts of intense exercise (140% VO_2 max) to exhaustion (MacDougall, Ward, Sale and Sutton, 1977).

Oxygen consumption during recovery from exercise

During passive recovery from exercise, oxygen consumption does not return to resting levels immediately but remains elevated for some time, indicating that there is still increased metabolic activity in muscle and other organs and tissues of the body, which may add significantly to the total energy cost of the exercise. The amount of oxygen consumed in excess of the resting value during the recovery period is referred to, in the classical concept, as the 'oxygen debt' and it was originally thought that lactate metabolism gave rise to that phenomenon (Hill, Long and Lupton, 1924). In 1933, Margaria, Edwards and Dill, modified this hypothesis by distinguishing between an initial, fast O_2-debt curve component (half-time approximately 20-30s) followed by a second, slow-component approaching the pre-exercise oxygen uptake level asymptotically. Using running protocols of 3-10min duration and observing the decline in blood lactate concentration during recovery, Margaria and co-workers (1933) hypothesised that the fast phase of the post-exercise oxygen consumption was due to rephosphorylation of ATP and PCr ('alactacid' in origin) and only the slow phase was linked to lactate metabolism ('lactacid' in origin).

These traditional views have been challenged and the actual function of the O_2-debt is now believed to be more complex than originally thought. Several studies, have demonstrated that the kinetics and magnitude of

the post-exercise oxygen uptake are independent of variation in the concentration of blood lactate and a time dissociation between these two variables exists. Segal and Brooks (1979) manipulated pre-exercise glycogen levels to show that glycogen-depleted subjects had significantly lowered blood lactate concentrations during and after exercise but had unaltered post-exercise oxygen consumption compared with glycogen-sufficient controls. Similarly, Roth, Williams, Stanley and Brooks (1988) increased blood lactate concentration by circulatory occlusion of the exercising limbs but found no significant effect on the slow component of the post-exercise oxygen uptake curve. Moreover, it has been demonstrated recently, that the metabolic changes observed in muscle, after one-leg exhaustive cycling exercise lasting 3.2min could account for less than one-third of the observed leg O_2-debt for the first 3min of recovery and this discrepancy was not reduced in the remaining 57min of the recovery period (Bangsbo et al, 1990). In the same study, resynthesis of PCr and ATP accounted only for about 20% of the observed O_2-debt during the first 10min of recovery but for the entire hour of recovery it accounted for less than 10% of the total oxygen consumption. It becomes apparent therefore, that in addition to lactate clearance and rephosphorylation of ATP and PCr, there are also other factors which influence metabolism during recovery.

During exercise oxygen stores of the body (i.e. myoglobin, dissolved oxygen in tissue fluids and haemoglobin in venous blood) become greatly reduced and a portion of the O_2-debt is required for reloading these stores. Restoration of ionic balance across muscle membrane is also required. Several studies have demonstrated dramatic changes particularly in K^+ concentrations within intracellular and extracellular fluids during high-intensity exercise (Bergström, Guarnieri and Hultman, 1971; Sjögaard, 1983; Sejersted, Medbo, Orheim and Hermansen, 1984; Sejersted and Medbo, 1989; Vollestad and Sejersted, 1989; Heigenhauser, Kowalchuck, Lindinger and Jones, 1989). For instance, it has been reported that following maximal isokinetic cycling lasting only 30s, the K^+ loss from the muscle was so large that extracellular K^+ concentration reached a value of $8.2 mmol.l^{-1}$ immediately post-exercise (McKenna, Heigenhauser, McKelvie, Sutton, MacDougall and Jones, 1989). The decline in plasma electrolytes concentration following high-intensity

exercise is relatively fast (half-time 2-3min) (Sejersted et al, 1984; Medbo and Sejersted, 1985; Vollestad and Sejersted, 1989). As restoration of ionic gradients are carried out by the Na^+/K^+ pump utilising chemical energy from ATP, it is evident that this active transport mechanism contributes significantly to elevated oxygen consumption during the recovery period. It has been suggested that the rapid restoration of ionic balance when exercise has ceased, is due to a significant increment of the Na^+/K^+ pump rate (Sejersted and Hallen, 1987).

In addition, the elevated plasma catecholamines will also contribute to oxygen consumption over time during recovery (Gaesser and Brooks, 1984). Recent studies have reported peak levels of plasma catecholamines to occur immediately post-exercise of maximal intensity but their pattern of elimination from the circulation seems to progress in a curvilinear fashion consisting of two components; an initial fast decline (half-time 1-2min) followed by a second, slow phase (Brooks et al, 1988; Allsop et al, 1990). The initial phase probably reflects the uptake by post-ganglionic neurones of the sympathetic nervous system, whereas the slow phase, which may take up several minutes (Allsop et al; 1990), probably is linked to the uptake and metabolism by other tissues (Lehman, Kapp, Himmelsback and Kuel, 1983 cited in Wootton, 1984 p34).

Temperature, has also been postulated as a possible mechanism for the exercise-induced increase in oxygen uptake at rest during recovery after exercise (Brooks, Hittelman, Faulkner and Beyer, 1971). It has been shown, that the decline in the post-exercise oxygen consumption was associated with the return of tissue temperature to control levels during the first hour of recovery following submaximal exercise (Brooks et al, 1971). Similar results have also been reported in another study (Chad and Wenger, 1986) but when measurements were continued until both variables had returned to resting levels, the decline of temperature was reached before resting oxygen consumption. It has been demonstrated that after 30s of maximal running muscle temperature was still increased significantly above resting levels (Allsop et al, 1990) although, post-

exercise oxygen consumption for the same type of exercise, has been shown to decrease to near resting levels in approximately 20min (Cheetham, 1987).

It is evident therefore, that the distribution of ionic gradients within the muscle and other body compartments, the effects of hormones released during exercise and elevated body temperature may continue to effect metabolism for some time in the recovery period. In essence, all of the physiological systems which are activated to meet the demands of exercise, also increase their own particular oxygen requirements in recovery.

2.6 METABOLIC PROFILE OF TYPE-I AND TYPE-II FIBRES IN HUMAN SKELETAL MUSCLE

Human skeletal muscle is composed of a mixture of two main muscle fibre types histochemically classifiable as type-I (slow-twitch) and type-II (fast-twitch) fibres. One striking feature of human skeletal muscle is the inter-individual variation in the proportion of type-I and type-II fibres within any given muscle. Although there is a wide range in the distribution, the proportion of type-II fibres in the vastus lateralis muscle of physically active, though not competitive individuals, has been found to average 53-59% (Tesch, 1980; Edström, Hultman, Sahlin and Sjöholm, 1982).

Although there appears to be no difference in glycogen content between the two fibre types of human skeletal muscle (Gollnick, Piehl, Saubert, Armstrong and Saltin, 1972b; Essén and Henriksson, 1974), divergent results have been presented in the literature with regard to the basal levels of PCr, in different muscle fibre types. Some reports observed higher resting PCr levels in type-II than type-I fibres (Essén, 1978; Tesch, Thorsson and Fujitsuka, 1989) or in vastus lateralis compared with soleus of human muscle (Edström et al, 1982). By contrast, the PCr content of distance runners was greater in type-I than type-II fibres (Rehunen, Naveri, Kuoppasalmi and Harkönen, 1982).

These two main fibre types exhibit different contractile characteristics as indicated by the activity of 'contractile enzymes' and their isozymes. Higher activities, almost twice as high, of Mg^{2+}-stimulated ATPase, creatine-kinase and adenylate-kinase have been found in pooled single type-II fibres than in type-I fibres (Thorstensson, 1976; Thorstensson, Sjödin, Tesch and Karlsson, 1977; Borges and Essén-Gustavsson, 1989). In addition to superiority in terms of contractile characteristics, type-II fibres display higher activity of enzymes thought to be rate-limiting for glycolysis. It has been shown that phosphorylase (a+b) activity is two or three times higher in type-II than in type-I fibres obtained from the quadriceps femoris muscle of humans (Harris, Essén and Hultman, 1976). Similarly, PFK activity in type-II fibres was found to be twice the level in type-I fibres whereas

succinate dehydrogenase (SDH) was increased by 50% in the type-I fibres. Moreover, a statistically significant correlation has been reported between LDH(tot) ($r = 0.96$) as well as M-LDH activity ($r = 0.98$) and fibre type distribution (Tesch, 1980). It appears therefore, that type-I and type-II fibres in human quadriceps muscle have distinctly different metabolic profiles as indicated by the activity of enzymes involved in aerobic and anaerobic metabolism. Thus, the greater potential of ATP hydrolysis in type-II fibres is matched by the higher activity of enzymes serving to catalyse reactions providing rapid resynthesis of ATP. For instance, greater PCr depletion and greater lactate accumulation have been shown in type-II fibres during a single bout of repeated maximal voluntary knee extensions (Tesch, 1980; Tesch et al, 1989) or during isometric contractions to fatigue (61% MVC) (Sahlin and Henriksson, 1984). In contrast, type-I fibres are best suited for use in prolonged activities with reliance upon oxidative metabolism (Essén, Jansson, Henriksson, Taylor and Saltin, 1975). However, whereas submaximalprolongedexercise has been shown to recruit type-I fibres, exhaustive sprint type activities recruit both type-I and type-II fibres as shown by the glycogen depletion from both fibre types (Jacobs, Kaijser and Tesch, 1981). Nevertheless, an earlier recruitment of type-II fibres during this type of exercise has been suggested (Gollnick, Armstrong, Sembrowich, Shepherd and Saltin, 1973).

Skeletal muscle with a high proportion of type-II fibres shows a greater tendency to fatigue compared to that with a high proportion of the oxidative type-I fibres. Indeed, several studies have demonstrated a positive relationship between the individual percentage of type-II fibres in the vastus lateralis muscle and the decrease in force output during one bout of repeated voluntary maximal knee extensions at a constant angular velocity (Thorstensson, 1976; Tesch, 1980; Tesch and Wright, 1983). Moreover, it has been shown that recovery in force after a single bout of maximal contractions (Tesch, Wright, Vogel, Sharp and Sjödin, 1983) or repeated bouts (Golliander, Dudley and Tesch, 1988), was positively correlated with a high proportion of type-I fibres. Although it has been shown that the rate of PCr resynthesis after maximal exercise may also be greater in type-I fibres, it is not as yet

conclusive whether the lower rate of force recovery demonstrated in individuals with a high proportion of type-II fibres correlates with PCr metabolism (Tesch et al, 1989).

Recent studies have shown that muscle buffering capacity may increase by proper training (Sharp, Costill, Fink and King, 1986) and that sprint-trained athletes exhibit a higher buffering capacity compared to marathon runners or untrained individuals (Parkhouse, et al, 1983; Sahlin and Henriksson, 1984). Although a higher concentration of the dipeptide carnosine, which is a potent intracellular buffer substance, has been observed in the quadriceps femoris muscle of sprint-trained individuals (Parkhouse et al, 1983), no statistical correlation has yet been found between buffering capacity and fibre type occurrence. It has been suggested that muscle which possesses a high glycolytic capacity may have a higher buffering capacity due to a higher content of PCr (Edström et al, 1982).

It appears therefore, that the variability in metabolic responses that is often observed during high-intensity exercise may be partially related to the inter-individual variation in muscle fibre composition.

2.7 METABOLIC ASPECTS OF FATIGUE DURING HIGH-INTENSITY EXERCISE

During high-intensity exercise a point is invariably reached when power output begins to decline. This decline in power output is usually referred to as muscle fatigue (Cheetham, 1987). The mechanisms by which exercise produces muscle fatigue under such conditions is unclear and several possible sites for fatigue have been proposed, from the motor cortex to the contractile apparatus itself. Fatigue may thus result from failure of central nervous system command (i.e. central fatigue) or peripheral mechanisms located within the contracting muscle (i.e. peripheral fatigue) (Edwards, 1981). The distinction between central and peripheral fatigue has been demonstrated by comparing the force generated by a maximal voluntary contraction with that of an electrically stimulated contraction (Bigland-Ritchie, Jones, Hosking and Edwards, 1978). Muscular contraction induced by electrical stimulation of a peripheral nerve (Edwards, Hill, Jones and Merton, 1977) or percutaneously via nerve endings in the muscle (Hultman and Sjöholm, 1983a) minimise the possibility of central fatigue because a constant stimulus can be delivered to the muscles independent of volitional effort, unlike dynamic exercise where the subject voluntarily exercises to exhaustion and therefore a reduced central motor drive could partly explain the fatigue process. It has been shown however, that in well-motivated subjects additional stimulation by interpolating twitch stimuli to muscles already undergoing voluntary contractions, did not result in additional force generation (Chapman, Edwards, Greig and Rutherford, 1984). Some studies have also demonstrated a parallel reduction in maximal voluntary force and maximal force produced by direct stimulation of the muscle (Bigland-Ritchie, Furbush and Woods, 1986; Merton, 1954). It appears therefore, that at least a substantial component of fatigue can always be found in the muscle, irrespective of the mode of stimulation. Moreover, because of the extremely high rates of energy turnover during high-intensity exercise, fatigue has been related to the metabolic changes within the muscle. In experiments on isolated frog sartorius muscle undergoing isometric contractions under anoxic conditions, it has been shown by NMR that the decline in force development was closely related with metabolite levels (i.e. increase in free ADP and H^+) and was proportional to the rate of ATP hydrolysis

(Dawson, Gadian and Wilkie, 1978). Similar metabolic changes (i.e. lactate accumulation and depletion of PCr) have also been observed on human muscle after short-term high-intensity exercise, in numerous studies employing both the biopsy and NMR techniques. Whilst the association of metabolite changes with fatigue seems to be clear, the further implication that it is the cause of fatigue is less certain. Even if such a causal relationship is present, the mechanism and the site where this might occur are not known. Two main theories of fatigue exist. Firstly, it has been suggested that the accumulation of metabolic products could directly inhibit the contraction process and thus reduce the demand for ATP. Alternatively, it has been hypothesised that the capacity to regenerate ATP could become insufficient for the demand, raising the possibility that energy cannot be exchanged at a sufficiently rapid rate to maintain high ATP turnover rates and thus, to permit muscular activity to continue without fatigue. Tissue acidosis and lactic acid accumulation have occupied central positions in both theories of fatigue. That H^+ accumulation plays an important role in the development of muscular fatigue becomes more evident from animal experiments in which acidosis was induced by two totally independent ways but the resulting mechanical response was similar. Acidosis induced in isolated rat muscle by incubation in CO_2 (Sahlin, Edström and Sjöholm, 1983) resulted in a similar decrease in isometric contraction force as when the decrease in muscle pH was achieved by electrical stimulation (2Hz) under anaerobic conditions (Sahlin, Edström, Sjöholm and Hultman, 1981). The increased concentration of H^+ could impair the contractile mechanism either directly or indirectly through an inhibitory effect on the ATP-generating processes. Thus, the possible mechanisms by which changes in metabolite levels and in particular H^+ concentration may contribute to fatigue during high-intensity exercise will be reviewed.

Evidence in support of the view that fatigue may be primarily due to inhibition of the contractile process, is provided from studies on skinned frog (Fabiato and Fabiato, 1978) and rabbit skeletal muscle fibres (Donaldson and Hermansen, 1978). The skinned muscle fibre preparation is an experimental technique by which the force-generating apparatus is functionally isolated from the activating steps and

therefore, the effect of chemical changes (such as H^+ and Ca^{2+}, determined by the composition of the bathing solution) on the development of tension can be studied at the site of cross-bridge formation. The results of these studies have shown that decreased muscle pH to values observed in fatigued muscle, lowered the maximum tension development at saturating Ca^{2+} concentrations and increased the requirement of Ca^{2+} to develop half-maximum tension. These effects were suggested to be caused either by a decreased sensitivity of the myofilament to Ca^{2+} or by increased Ca^{2+}-binding by the sacroplasmic reticulum at low pH and were most prominent in type-II fibres with the same degree in pH (Donaldson and Hermansen, 1978). A decreased Ca^{2+} sensitivity of the system could be attributed to a competition of H^+ for activating Ca^{2+}-sites, but since saturating Ca^{2+} concentrations were used in both studies this mechanism would not explain the acidotic depression of maximal force generation, at least in vitro. It has been suggested, that the most likely explanation is that the increase in H^+ concentration causes a product inhibition of the actomyosin ATPase thus, lowering force generation (Donaldson, 1983). This suggestion also raises the possibility that acidosis may reduce the free energy yield per molecule ATP hydrolysed to level below that required to maintain cross-bridge cycling (Dawson et al, 1978). Indeed, it has been calculated that the amount of energy liberated, when one mole of ATP is hydrolysed to ADP, decreased from 54kJ at rest to 50kJ after exhaustive dynamic exercise and assuming that IMP formation did not occur during exercise the energy liberated was estimated to be $46kJ.mol^{-1}$ ATP (Sahlin et al, 1978). Although the minimum energy which is required to break and construct a new bond between myosin and actin is not known, a threshold value of about $40kJ.mol^{-1}$ has been suggested, below which cross-bridge cycling becomes impossible (Dawson et al, 1978).

One way by which the effect of H^+ accumulation could be separated from other metabolic changes in fatigued muscle is iodoacetate (IAA) poisoning which inhibits glycolysis and lactic acid production. It has been demonstrated that electrical stimulation of unpoisoned rat muscle (2Hz for 3min) resulted in lactic acid formation (15-fold) and decrease of muscle pH ($\Delta pH = 0.34$), in parallel with a decrease in tension development (to 50% of initial value) and an increase in relaxation time

(to 250% of initial). In contrast stimulation of IAA-poisoned muscles for one minute decreased tension to 50% but resulted in unchanged muscle pH and relaxation time (Sahlin, Edström, Sjöholm and Hultman, 1981). As accumulation of lactic acid is the most obvious difference between IAA-poisoned and unpoisoned rat muscles, it would seem likely that H^+ is also involved in the slowing of the relaxation process. This would impair the performance in dynamic exercise where a well-timed rhythm of contraction and relaxation of muscles is required. It has been suggested that when muscle fatigues the rate of relaxation is slowed down either directly through a pH effect on the Ca^{2+} uptake by the sarcoplasmic reticulum or indirectly by combination with other factors such as accumulation of ADP (Sahlin et al, 1981). In the muscle cell ADP is composed of several ionic species and therefore a decrease in muscle pH would increase the protonated forms of ADP which might be of importance in the relaxation process (Sahlin, 1978). A progressive increase in the relaxation time has also been demonstrated during electrical stimulation (20Hz) of human quadriceps femoris muscle with occluded circulation to the legs and was found to increase by about 200% after 75s of stimulation (Hultman, Sjöholm, Sahlin and Edström, 1981). A rapid normalisation of the relaxation time was noted however in that study during the recovery period, when the circulation was restored, at approximately the same rate of PCr resynthesis.

This observation further raises the question that if there is a direct relationship between H^+ accumulation and force depression, as suggested in studies employing the skinned fibre technique or IAA-poisoning, then one should find a close parallelism between the restoration of force and pH during the recovery period. In vitro studies on quite a different preparation, the rat diaphragm, have shown that following high (75Hz, for 1min) and low (5Hz for 1.5min) frequency stimulation, there was a high correlation between recovery of force and intracellular pH recovery, during the first 10min, suggesting that the slow phase of force recovery is at least partially linked to an H^+-mediated effect in excitation-contraction coupling (Metzer and Fitts, 1987). Furthermore, it appears that recovery of force follows a time-course which is very dependent on the external pH of the medium and it is little influenced by the extent of fatigue. It has been demonstrated in isolated rat

diaphragm (Mainwood and Alward, 1982) and frog muscle (Mainwood and Renaud, 1985) that following tetanic stimulation, the recovery of force development was inhibited if the external pH was low (6.2-6.4) but was restored rapidly if extracellular pH was raised (7.4). Inhibition of force recovery at low external pH, which has been associated with a slow rate of H^+ efflux (Mainwood and Worsley-Brown, 1975), would suggest direct involvement of H^+ in the recovery of force. Recently, in ^{31}PNMR studies of the adductor pollicis muscle on humans, a high relationship has been shown between accumulation of H^+ and decline of force during sustained and intermittent voluntary isometric contractions (Miller et al, 1988). Despite this, an initial rapid recovery in force from 30% to 80% MVC occurred during the first minute of recovery whereas no recovery occurred in muscle pH. Similar observations have been reported in another study which showed that despite a low muscle pH (calculated), contraction force (half-time less than 15s) was completely restored after two minutes of recovery in human quadriceps femoris muscle (Sahlin and Ren, 1989). In contrast, time to fatigue (from 66% to 50% MVC) recovered more slowly (half-time approximately 1.2min) and was still significantly depressed after four minutes of recovery. Conclusively, both studies (op sit) indicate that acidosis did not impair contractile force but limited endurance during a sustained contraction, possibly indicating that the relationship between H^+ accumulation and the fatigue process is due to a reduced energy supply via an H^+-mediated inhibition of the ATP-generating processes.

Recently, it has been proposed, on the basis of ^{31}PNMR observations in frog (Wilkie, 1986) and human muscle (Wilson et al, 1988) that fatigue is not due directly to a high H^+ concentration but rather is primarily due to production of the diprotonated acidic form of Pi ($H_2PO_4^-$). This is supported by a close relationship that was found between $H_2PO_4^-$ and force during the development of fatigue in these studies. In the latter study, in particular, when maximal exercise (wrist flexion) was preceded by submaximal exercise, marked changes in pH were noted but this situation did not produce more rapid fatigue during maximal exercise compared with control (Wilson et al, 1988).

It is also known from studies on humans that K^+ is lost from the muscles during both high-intensity dynamic and isometric exercise (Sjogaard, 1986). Activation of muscle is critically dependent upon the extracellular electrolyte composition and increases of K^+ may result in reduced excitability of the sarcolemma and contribute to fatigue (Sejersted et al, 1984). Many investigations point to an important relationship between changes in intracellular pH and K^+. The classical concept is that K^+ is expelled from muscle cells when intracellular pH is lowered (Burnell, Villamil, Uyeno and Scribner, 1956). It has been shown however, that the decline in extracellular K^+ concentration during recovery following intense exercise is faster when compared with the intracellular pH recovery (Saltin, Sjogaard, Strange and Juel, 1987). This suggests that intracellular K^+ recovery may also be taking place whilst the intracellular pH is still low. It is therefore unlikely, that the K^+ displacement is regulated by pH. It has been suggested, that a net K^+-loss from the intracellular space could be due to an insufficient Na^+/K^+-pump activity relative to the action potential induced Na^+/K^+ exchange (Clausen, Everts and Kjeldsen, 1987).

It appears therefore, though not conclusively, that there are several mechanisms by which accumulation of metabolites might exert directly their negative effect on force generation, and therefore on ATP hydrolysis for muscular contraction. However, direct involvement of H^+ accumulation in the contractile mechanism is not obligatory for the development of fatigue. It has been shown that muscles of patients with phosphorylase deficiency and unable therefore to produce lactic acid during ischaemic exercise, fatigued even more rapidly than normal muscles (Wiles, Jones and Edwards, 1981). In addition, rat muscle treated with iodoacetate, depleted its ATP store and ended up in rigor (Sahlin et al, 1981). Under these conditions it appears that fatigue is the result of an insufficient rate of ATP resynthesis. In contrast, in muscles with intact glycogenolysis, the cause is less obvious because both muscle biopsy and NMR studies have generally failed to show any major depletion of this metabolite during exercise.

Inadequate availability of ATP in myofibrillar regions has been suggested as a possible cause of impaired contractile function in frog and rat muscle (Fitts and Holloszy, 1976; Fitts and Holloszy, 1977). However, the evidence from NMR studies in frog muscle (Brown, Chance, Dawson, Gadian, Radda and Wilkie, 1980) and IAA-poisoned rat muscle (Sahlin et al, 1981) have indicated that ATP is not metabolically or spatially isolated but exists in a pool available for muscle contraction. In addition studies on skinned muscle fibres have shown that the force generation was not dependent on ATP concentrations as low as 0.03M (Donaldson, Bond, Seeger, Niles and Bolles, 1981). It is unlikely therefore, that the muscle content of ATP per se could be the limiting factor for tension development in muscle. The question then, may be raised whether or not the decrease in the muscle ATP store could be restricted to just one type of muscle fibres. If so, selective depletion of ATP stores in muscle fibres could explain why total analysis has failed in most cases to show the expected correlations between depletion and the onset of fatigue. Indeed, fibres devoid of ATP have been identified in equine muscle after exercise, but these have been attributed to experimental error (Snow et al, 1985). It has been reported that deamination of AMP to IMP occurred rapidly in type-II but not in type-I muscle fibres of rats during intense stimulation or strenuous exercise (Meyer, Dudley and Terjung, 1980; Meyer and Terjung, 1979) indicating, possibly, that the activity of AMP-deaminase is higher in type-II than type-I fibres. Moreover, it has been shown in rat (Dudley and Terjung, 1985) and human muscle (Harris and Hultman, 1985) that during exercise IMP accumulation did not occur until muscle lactate content reached 50-75mmol.Kg^{-1}dm. This suggests that intracellular acidosis is important for the activation of AMP-deaminase, especially in type-II fibres (Dudley and Terjung, 1985). It should be noted, that some studies have shown that acidosis may not be a prerequisite for activation of AMP-deaminase, in vivo (Katz, Sahlin and Henriksson, 1986a; Broberg and Sahlin, 1989). Thus, although fatigue could result from a selective depletion of ATP in different fibres, effectively removing them from the total available for recruitment (Harris, 1985) it would seem unlikely that the body's integrative mechanisms would allow irreversible cell damage to occur.

A limitation to ATP resynthesis is obviously the availability of PCr. However, the consequence of a low PCr concentration is also unclear. In human studies, the decline in maximal power output is noted within the first few seconds of maximal dynamic exercise (Wooton, 1984; Cheetham et al, 1986), possibly suggesting that the tremendously high rates of ATP resynthesis required to sustain such a high power output, could only be maintained for a few seconds. Indeed, under such conditions most of the PCr store is utilised, though not depleted, and a small decrease in ATP concentration is also observed (Boobis et al, 1982; Hirvonen, Rehunen, Rusko and Harkonen, 1987). However, during intermittent maximal isokinetic cycling, the ensuing maximal power output was reported to be substantially less in successive bouts than in the initial exercise period (30s) although resynthesis of PCr was almost complete in the recovery period (4min), (McCartney et al, 1986). This is surprising because PCr degradation is the most rapid process in ADP rephosphorylation (Sahlin, 1986) and therefore, it is unlikely that even if an inhibition of glycolysis had occurred, the capacity to generate maximal power output should not be impaired. It appears therefore, that the content of PCr store in muscle might not be the most important contributory factor to fatigue during brief maximal dynamic exercise. Moreover, impairment in force generation has been shown during brief maximal isometric contractions in human quadriceps muscle before the whole PCr store is utilised and practically the whole ATP store still close to resting values (Bergström et al, 1971; Hultman and Sjöholm, 1983a). Studies using the NMR-technique in isolated frog muscle have also shown that isometric force development was not proportional to PCr concentration (Dawson et al, 1978). It has been suggested that in these conditions fatigue may be due to inhibition of ATP utilisation rather than to insufficient formation (Hultman et al, 1987; Spriet et al, 1987b).

If the exercise is prolonged to 30-60s considerable accumulation of lactate and depletion of PCr stores occurs (see Section 2.3). Accumulation of H^+ during such intense exercise conditions could produce a decrease in muscle pH, large enough to decrease the rate of anaerobic glycogen degradation by inhibiting phosphorylase and PFK activity. Several studies have indeed demonstrated reduced glycogenolytic and

glycolytic rates during high-intensity exercise. Moreover, a decreased muscle pH, could cause a further decrease of the PCr levels due to the involvement of H^+ in the creatine-kinase equilibrium. The regulation of both mechanisms during exercise has already been reviewed (Section 2.3). Although depletion of PCr would reduce the ATP turnover rate and thus could limit the ability to sustain further exercise, it has been reported that during sustained ischaemic contractions the ratio of ATP turnover to force was gradually reduced, but without affecting force development (Hultman and Sjöholm, 1983a; Spriet et al, 1987b). Thus, despite a reduced rate of ATP supply under these conditions, development of force was maintained due to an increased economy of muscular contraction.

In addition the importance of a decreased muscle pH on the supply of ATP from anaerobic glycogen degradation is also questionable since significant glycogenolytic and glycolytic activity has been reported during electrically stimulated ischaemic contractions (20Hz) at low pH (Spriet et al, 1987b). It has been demonstrated that inhibition of PFK may be overcome by increased AMP, ADP levels and increased contractions of certain glycolytic intermediates (see Section 2.3). However, it should be noted that in these studies (Hultman and Sjöholm, 1983a; Spriet et al, 1987b) force production is considerably less of that generated during maximal voluntary contractions and thereforeitis possible that in dynamic exercise the situation is different. For instance, in the study by Spriet and co-workers (1987b) pH decreased to 6.4 following 102.4s of electrical stimulation but after 25.6s muscle pH was 6.7 similar to pH values observed after 30s of maximal dynamic exercise (Cheetham, 1987). However, isometric force production was only marginally reduced at a pH of 6.7 whereas after 30s of sprint running or cycling, power output is known to be reduced by 50% (Cheetham et al, 1986; Wootton, 1984). These observations seem to suggest that during maximal dynamic exercise the inability to maintain high ATP turnover rates may be the most contributory factor to fatigue.

It appears therefore, that when the capacity to rephosphorylate ADP is diminished a decrease in the ATP concentration mediated via the activity of adenylate-kinase and AMP-deaminase is observed. However, the

formation of lactic acid or other by-products prevents apparently a pronounced ATP depletion, that would otherwise cause rigor development, when the energy stores of glycolysis and PCr have reached their limits (Wilkie, 1981; Harris, 1985). This may be interpreted as indicating that the primary cause of fatigue is due to insufficient ATP formation.

2.8 EFFECT OF INDUCED ALKALOSIS ON PERFORMANCE DURING HIGH-INTENSITY EXERCISE

The onset of fatigue during short-term intense exercise has been attributed, in part, to acidosis as a result of H^+ accumulation in the muscle cell. An increase therefore, in muscle buffering capacity or in the rate of H^+ efflux from muscle to the circulation should delay the onset of fatigue.

A number of studies have attempted to increase buffering capacity by giving strong doses of buffering substances prior to exercise (Table 2.9). However, the results of these studies are both conflicting and inconclusive. Nevertheless, there have been reports of longer working times to exhaustion lasting more than five minutes after inducing alkalosis using sodium bicarbonate ($NaHCO_3$) (Jones, Sutton, Taylor and Toews, 1977; Sutton, Jones and Toews, 1981). The ergogenic effect of $NaHCO_3$ has also been demonstrated in field studies. Wilkes and co-workers (1983) reported that trained track athletes had significantly faster times in an 800m race when they ingested $NaHCO_3$ prior to competition. The average improvement was 2.9s and represents a distance of 19m (Wilkes, Gledhill and Smyth, 1983). It has also been reported that the average 400m running time was improved by 1.52s after ingestion of $NaHCO_3$, which caused blood pH and standard bicarbonate concentration to be higher than in the placebo condition (Goldfinch, McNaughton and Davies, 1988). In contrast, no improvement in performance time was observed in a 400m run when alkalosis was induced by the infusion of bicarbonate or Tris-buffer, despite elevation of the buffering capacity of the blood by over 5mmol.l^{-1} and blood pH to 7.5 (Kinderman, Keul and Huber, 1977). The claim that the induction of pre-exercise alkalosis has no significant improvement on performance has also been supported by McCartney and co-workers (1983) who also demonstrated that the total work done over a 30s isokinetic cycle ergometer test (100rpm), after ingestion of $NaHCO_3$, was only 101% of that achieved with a placebo (McCartney, Heigenhauser and Jones, 1983). Similarly, alkalosis had no significant effect on a single bout of cycling exercise to exhaustion at 125% VO_2 max (Katz, Costill, King, Hargreaves and Fink, 1984).

The influence of 'bicarbonate loading' on performance during intermittent exercise has received relatively little attention in contrast with the studies on continuous high-intensity exercise. It has been shown that endurance time was improved by 42% in the fifth bout of exercise preceded by four, 1 min bouts of exercise at 125% VO_2 max (Costill, Verstappen, Kuipers, Jenssen and Fink, 1984). In the same study, ingestion of bicarbonate resulted in a post-exercise muscle pH of 6.81 compared to 6.73 in the control condition. Employing an identical exercise protocol Winjen and co-workers (1984) observed only a modest and non-significant improvement in endurance time (Winjen, Verstappen and Kuipers, 1984). Additionally, Parry-Billings and MacLarren (1986) failed to observe any significant differences in the maximal power output and the total work done over three 30s bouts of maximal cycling exercise, despite the significant shifts in acid-base balance of the blood that occurred as a result of $NaHCO_3$ or sodium citrate ingestion. Recently, it has also been shown that power output during exercise consisting of ten 10s sprints on the cycle ergometer, with 50s recovery between each sprint, was enhanced following induced alkalosis (Lavender and Bird, 1989).

It is recognised that administration of oral alkalising agents do not directly alter intramuscular pH prior to exercise (Adler, Roy and Relman, 1965). This is due to the relatively impermeable nature of the sarcolemma to bicarbonate (Robin, 1961). Thus, the enhancement of intramuscular buffer capacity prior to exercise as a result of sodium bicarbonate would appear to be unlikely. As ingestion of bicarbonate does not increase resting muscle pH and yet it has been shown to improve performance under certain exercise conditions, it would appear that an increased buffering potential of the blood enhances the efflux of H^+ from the working muscles into the circulation (Mainwood and Worsley-Brown, 1975; Hirche et al, 1975). It appears that in the studies of longer duration exercise but of lower intensities, the increased buffer capacity of the blood induced by ingestion of oral alkalising agents has sufficient time to facilitate the efflux of H^+ from the muscle, thereby lessening the drop in intramuscular pH. Additionally, it may be possible that the increased buffering capacity of the blood may facilitate H^+ efflux from the muscle during sufficient recovery

intervals and explain the pronounced improvement in performance that have been observed in some studies during repeated bouts of high-intensity exercise.

TABLE 2.9 Effect of induced alkalosis by oral ingestion of sodium bicarbonate (unless specified otherwise) on exercise performance.

REFERENCES	DOSE ($g.kg^{-1}bw$)	ALKALI ADMINISTRATION BEFORE EXERCISE (h)	TYPE OF EXERCISE	DURATION OF EXERCISE	PERFORMANCE INDEX	RESULTS % IMPROVEMENT OF PLACEBO
Poulus et al (1974)	0.3	0 (infusion)	Cycling[a]	>30min	Total work	b
Jones et al (1977)	0.3	3	Cycling 95% VO_2 max[c]	2.5-8.0min	Endurance	+62($p<0.01$)
Kinderman et al (1977)	0.2	0.25 (infusion)	400m run	1min	Endurance	b
Sutton et al (1981)	0.3	3	Cycling 95% VO_2 max[c]	5min	Endurance	+17($p<0.05$)
Inbar et al (1983)	10g(total)	3	Cycling max (WAnT)	30s	Mean power output	+1.2($p<0.05$)
Wilkes et al (1983)	0.3	2.5	800m run	2min	Endurance	+2.3($p<0.05$)
McCartney et al (1983)	0.3	3	Isokinetic cycling max 100rpm	30s	Mean power output	+2.2 (n.s.)
Costill et al (1984)	0.2	1	Cycling 125% VO_2 max[d]	1-2min	Endurance	+42($p<0.01$)
Wijnen et al (1984)	0.18/0.36	1	Cycling 125% VO_2 max[d]	2-2.5min	Endurance	+0.4-22 (n.s.)
Kowalchuck et al (1984)	0.3	3	Cycling[e]	19.4min	Endurance	b

TABLE 2.9 (continued)

REFERENCES	DOSE ($g.kg^{-1}bw$)	ALKALI ADMINISTRATION BEFORE EXERCISE (h)	TYPE OF EXERCISE	DURATION OF EXERCISE	PERFORMANCE INDEX	RESULTS % IMPROVEMENT OF PLACEBO
Parry-Bilings & MacLaren (1986)	0.3 0.3	2.5	Cycling max (WAnT)	3 x 30s (6min recovery)	Total work done	+1 (n.s.)
Knudsen & Pedersen (1987)	0.3	2-3	Cycling 80% VO_2 max	31-34min	Endurance	b
Bouissou et al (1988)	0.3	2	Cycling 125% VO_2 max	1.26min	Endurance	+20% (?)
Horswill et al (1988)	0.1 0.15 0.2	1 1 1	Isokinetic Cycling	2min 2min 2min	Total work	-0.5 (n.s.) -2.0 (n.s.) -0.2 (n.s.)
Gao et al (1988)	2.9[f]	1	Freestyle Swimming	5 x 91.4m (2min recovery)	Time	See Fig 1 ($p<0.05$) p.172
Goldfinch et al (1988)	0.4	1	400m Run	56.94s	Time	+2.9 ($p<0.01$)
Lavander & Bird (1989)	0.3	2	Cycling max	10 x 10s (50s recovery)	Peak Power output	+2.0 ($p<0.01$) (bout 10)

Note: a = incremental cycling increased by $10W.min^{-1}$ to exhaustion
b = no change in performance under bicarbonate treatment
c = following cycling for 20min at 33% VO_2 max plus 20min at 66% VO_2 max
d = exhaustive cycling followed 4 x 1min cycling bouts at 125% VO_2 max with 1min recovery
e = incremental cycling increased by $100kpm.min^{-1}$ to exhaustion
f = $2.9mmol.kg^{-1}bw$
Abbreviations: n.s. = not significant

2.9 SUMMARY

The evidence provided in this chapter has demonstrated that many parameters do change quite drastically during the development of fatigue but it appears that the qualitatively most important changes are those of the H^+ concentration. An increased H^+ concentration could influence force generation by interfering with ATP utilisation at the level of excitation-contraction coupling and/or reducing the rate of energy provision. It is possible therefore, as previously suggested, that the increase in H^+ concentration could provide a common regulator for the control of both the maximal rate of ATP utilisation and the maximal rate ATP may be resynthesised (Hermansen, 1981).

In the present thesis, the metabolic responses to intermittent maximal exercise are described. In addition the relationship between muscle metabolism and fatigue is examined by ingesting solution of sodium bicarbonate and by occluding the circulation to the legs during this type of exercise.

3. GENERAL METHODS

3.1 INTRODUCTION

In the present thesis, two intermittent maximal exercise tests performed on a friction-loaded cycle ergometer were used to examine fatigue. Specifically, in two studies (Study 1/Chapter 4 and Study 3/Chapter 6) the exercise test consisted of ten 6s sprints with 30s recovery between each exercise bout and is referred to as the 'multiple-sprint test'. In another study (Study 2/Chapter 5) the test consisted of repeated maximal exercise bouts with 30s recovery intervals but during each exercise period the amount of work performed was standardised and set to that attained by a prior single 6s maximal exercise bout. This test is referred to as the 'constant-work multiple-sprint test'. These performance tests are not novel in design and their development, validity and variability has previously been reported in detail (Wootton, 1984; Lakomy, 1988).

In this chapter the methodology common to all studies of the thesis is reported. In doing so, the methodology is divided into two sections. The first section deals, with the instrumentation of the equipment used to determine power output, the modifications made to the design of the exercise tests employed, and the standardised testing procedures used during administration of these tests. In the second section the procedures followed to obtain and analyse blood and muscle samples are described in detail.

3.2 THE MULTIPLE-SPRINT TESTS

Measurement of power output generated on the friction-loaded cycle ergometer

In the present thesis, all performance tests were carried out on an instrumented friction-loaded cycle ergometer (Monark, model 864), which allowed the corrected instantaneous power output and work performed during maximal exercise to be monitored and recorded, precisely. The design of this equipment has been described in detail, elsewhere (Lakomy, 1988). Briefly, the instantaneous changes in flywheel angular

velocity were constantly monitored with the use of a small electrical generator which was driven by the flywheel, giving an analog signal proportional to the angular velocity of the flywheel (r^2 = 99.8%). This signal was then logged, via an analog-to-digital (A-to-D) convertor, by a BBC (model B) microcomputer, along with a timing signal derived from the computer's internal clock. The sampling rate was restricted to 20Hz to reduce the magnitude of the error that might result from this relatively slow clock.

In order to calculate correctly the power output generated during maximal exercise it was assumed that the external mechanical work performed on this type of cycle ergometer was equal to the work dissipated against braking forces (i.e. inertia of the flywheel, rolling resistance of the flywheel and freewheel mechanism) plus the kinetic energy of the flywheel. Thus the 'effective load' on the cycle ergometer was determined. This is the load required to prevent acceleration of the flywheel and consisted not only of the frictional load that was applied to the flywheel, as is the case in the Wingate-test, but also took into account the frictional load that would be required at any instant to stop the subject from accelerating the flywheel. This is known as 'acceleration balancing load'. The instantaneous product of flywheel speed and effective load was used to determine corrected power output throughout each exercise bout. The equipment used for the maximal cycling performance tests is shown schematically in Figure 3.1.

Calibration of flywheel speed and determination of acceleration balancing load

Prior to each test the relationship between flywheel speed and voltage output from the generator and the A-to-D convertor was calculated. In doing so, following a warm-up period of 5 minutes, flywheel revolutions were counted for about two minutes at approximately 65 pedal revolutions per minute (rpm) using an electromechanical counter driven by an eccentric cam attached to the flywheel. Logging of the A-to-D reading

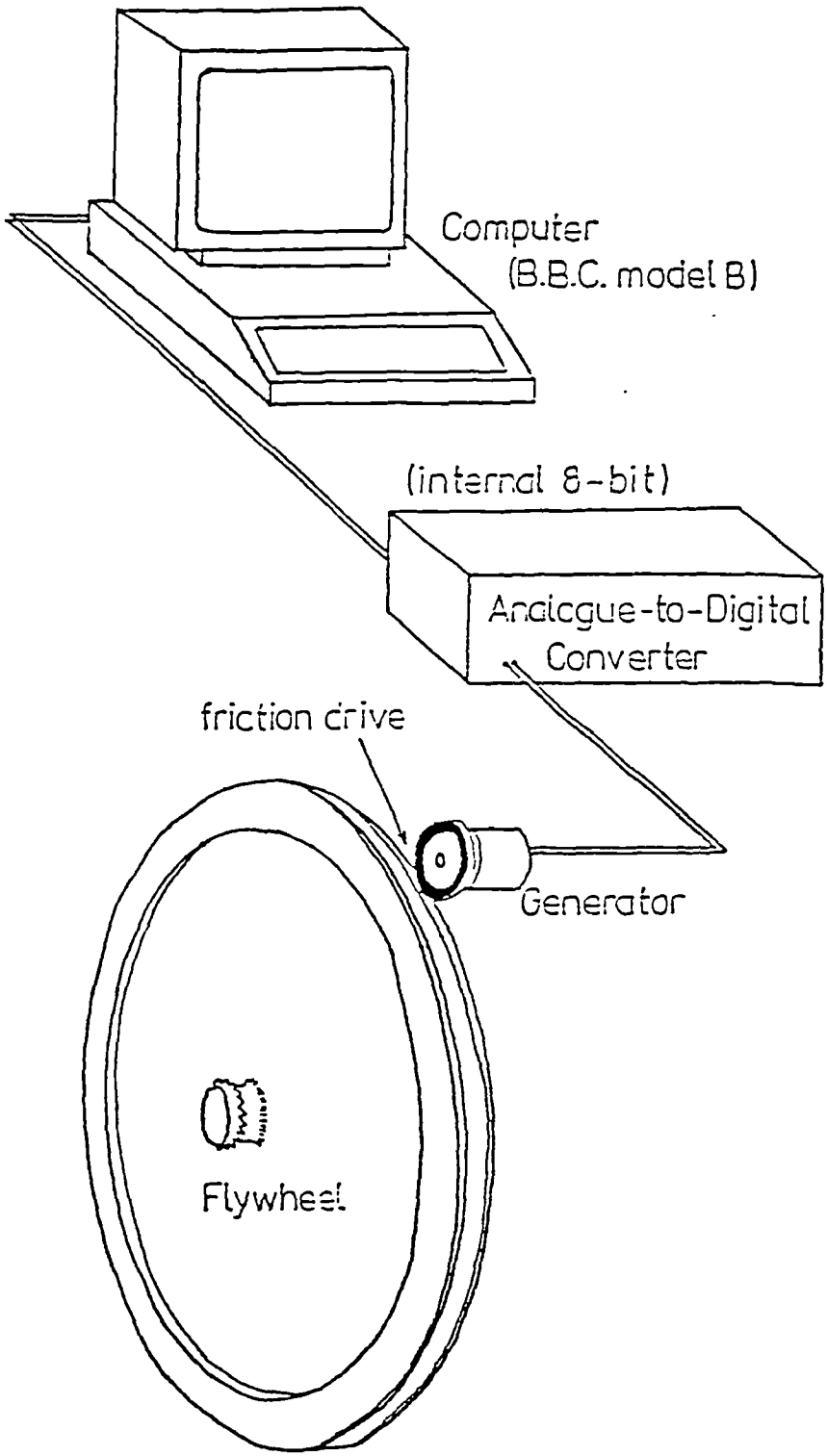


Figure 3.1. System used in the Monark basket-loaded ergometer for data collection (after Lakomy, 1988).

was started and stopped at the same time that the electromechanical counter was switched on and off. The average of the A-to-D readings was equated with the average speed of the flywheel and a conversion factor was obtained and stored on disc for retrieval by the test programs.

The determination of the acceleration balancing load required the generation of a set of deceleration curves using loads of known weights (the loads used were 0.5, 1.0, 1.5, 2.0 and 3.0Kg). If a given deceleration of the flywheel results from a given load, then the same load would be required to balance any torque attempting to accelerate the flywheel at the same rate. During this calibration routine, the deceleration curves were obtained by pedalling over 105 pedal rpm against each frictional load and recording only the deceleration phase resulting from cessation of pedalling (Figure 3.2). When the deceleration data were plotted against load a linear regression equation was obtained and was used to calculate acceleration of the flywheel for a known resistive load (Figure 3.3). The correlation coefficient for the regression line was calculated by the computer and an r^2 value of less than 99% was rejected and the entire procedure was repeated. The determination of the acceleration balancing load was carried out prior to each test because the characteristics of the cycle ergometer and A-to-D convertor can vary with temperature and state of maintenance. The error due to variations in the regression equations obtained from this cycle ergometer on three successive days has been calculated to be less than 1% (Lakomy, 1988).

Method of Initiating the Tests and Selection of Load Setting

In the protocol described by Bar-Or (1978) the subjects started pedalling as fast as possible against a low resistance which is increased to the required level during the first 2 or 3s of the Wingate Anaerobic Test. Many researchers have replaced this stationary start with a rolling start at a predetermined submaximal speed (usually at 65-75 rpm) with the maximum effort commencing once the required load has been introduced (Wootton, 1984; Lakomy, 1988).

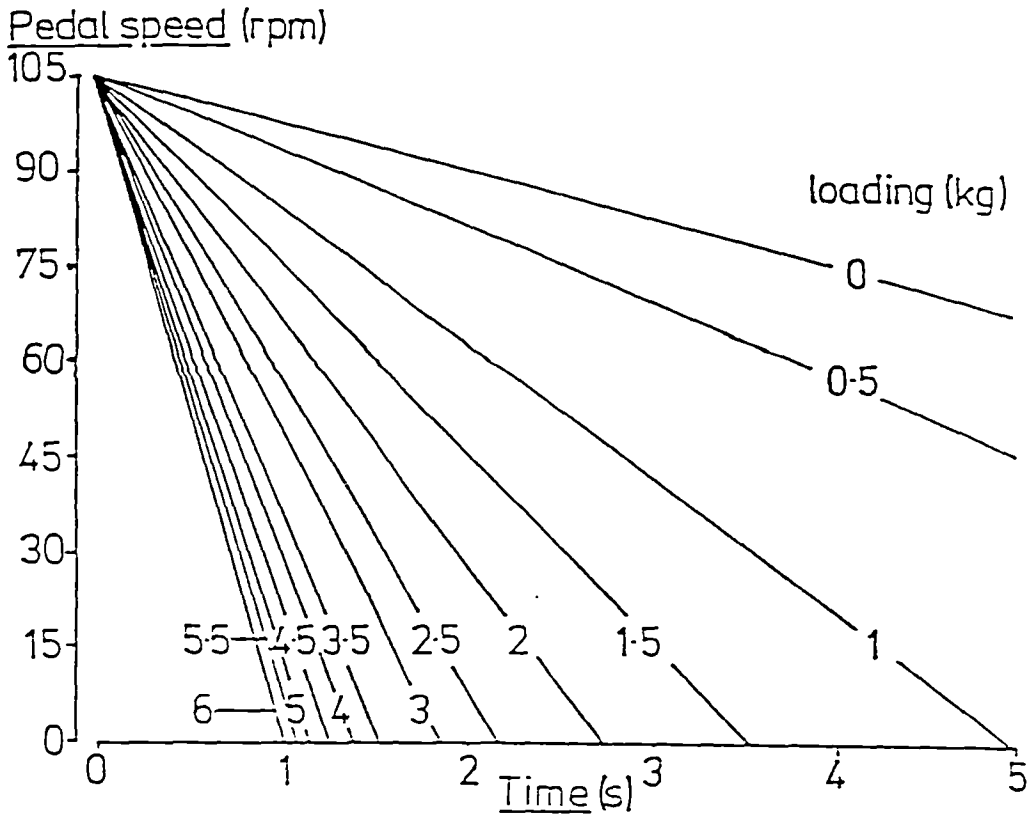


Figure 3.2. Flywheel deceleration curves obtained for the range of 105 to 0 pedal r.p.m. for loads of 0.5 to 6.0 kg (after Lakomy, 1988).

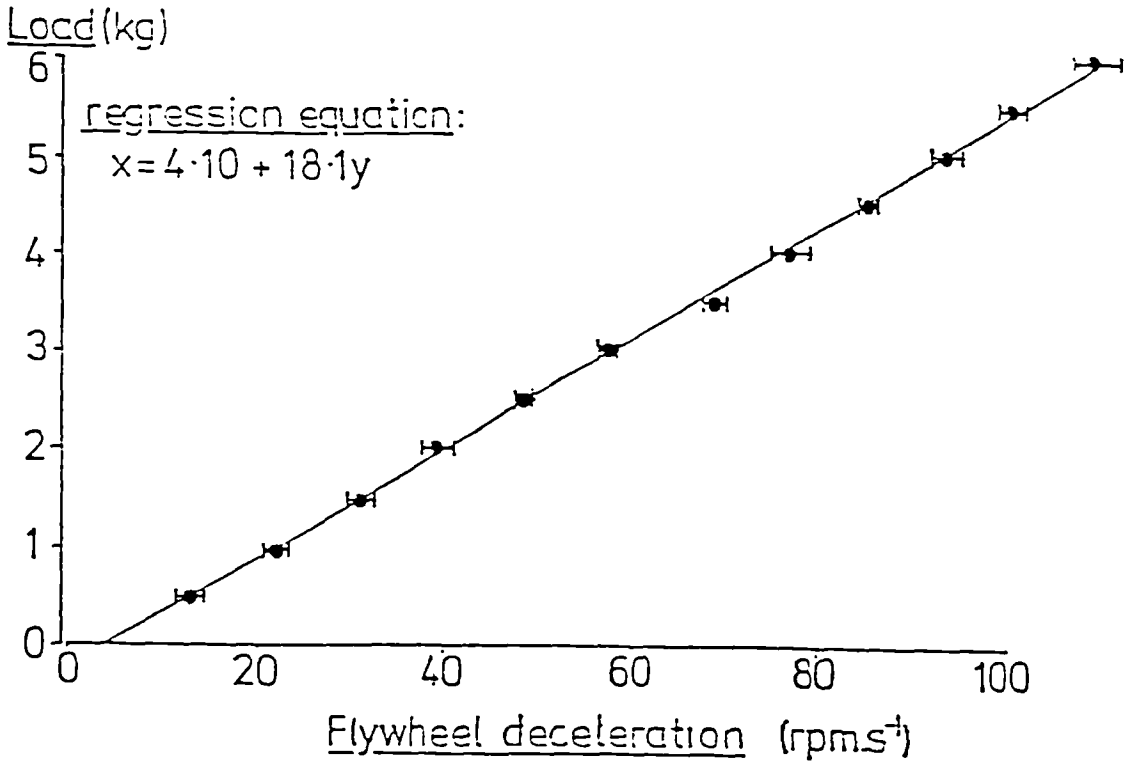


Figure 3.3. Plot of flywheel deceleration against load (after Lakomy, 1988).

A comparison of performance with rolling and stationary starts in a standard Wingate Anaerobic Test has shown that a stationary start elicited higher maximal power output to that obtained advocating a rolling start although the time taken to reach maximal power was considerably longer (6.0 $\pm$ 1.4 vs 3.0 $\pm$ 2.0 seconds, respectively), (Coleman, Hale and Hamley, 1985). This was demonstrated however, using the uncorrected method of power output calculation and collecting data every 0.4s. In contrast, it has been reported recently that time taken to achieve maximal power output was always shorter for the corrected power output values than for the uncorrected values, integrated over each 0.5s of the test (Lakomy, 1988). This was achieved in the former condition within the first 2s of maximal cycling exercise, irrespective of the mode the sprint was initiated. It was decided therefore to use the stationary rather than the rolling start in all studies reported in this thesis, because of the increased accuracy and ease with which the starting point could be determined by the computer. The plot of the instantaneous flywheel speeds achieved during each exercise bout were displayed on the computer's screen after termination of the test and a cursor line was moved on the screen until it coincided with the point at which a break in flywheel speed from zero could be seen. The computer was then instructed to regard this point as the start of each exercise bout. In addition, a standing start was favourable in the 'constant-work multiple-sprint test' because the calculation of the standardised amount of work was done in real time so that the subjects knew when to stop pedalling. In doing so, the computer was programmed (Lakomy, 1988) to wait until it detected movement of the flywheel. When this occurred, the internal clock of the computer was activated until the predetermined amount of work was achieved, at which point, the computer triggered a buzzer to indicate that the subject should stop pedalling.

The effect of different frictional loads (i.e. 65, 75, 95, 105, 115g.Kg^{-1} body weight) on the corrected maximal power output and time taken to achieve it from the start of the sprint has also been investigated (Lakomy, 1988). The results of that study showed that for corrected maximal power output, the lighter the load the greater the power output and the shorter the time taken to achieve it. However, such loads were suggested not to be suitable for optimum maximal mean

power output over a 30s maximal test, as they have been found to cause a greater decline in power output than heavier resistive loads. Owing to the brief duration of exercise bouts (i.e. 6s) used in the studies of this thesis, it was thought that a light load (55g.Kg^{-1} body weight) might have been more suitable for this type of exercise. However, the initial familiarisation session indicated that this load was very light because some subjects could not maintain maximal leg speed after the initial acceleration phase of the flywheel. When a heavier resistive load was introduced (75g.Kg^{-1} body weight), most subjects found the standing start extremely difficult and tended to rise from the saddle in the acceleration phase. The resistive load therefore used in all studies was set at 65g.Kg^{-1} body weight.

Selection of the Averaging Period for Calculation of Performance Indices

The instrumentation of the cycle ergometer allowed continuous sampling of instantaneous flywheel speeds over the exercise period. Selection of the averaging period was important because increasing the averaging interval could act as a smoothing process in the data collected. It has been shown that when the average sampling period was less than a pedal stroke duration (e.g. 0.25s) then within stroke variations were detected resulting in a multi-peaked plot (Lakomy, 1988). In contrast, an average sampling interval over 1s showed a smooth curve with a single distinct peak occurring within the first 2s of the sprint. Thus, for the purposes of the experiments reported in this thesis, following conclusion of the final sprint, results were integrated over 1s time intervals using the trapezoid rule and displayed by the computer. The following performance indices were obtained:

(a) the maximum 1s integral of effective load and flywheel speed, referred, to as peak power output (PPO) expressed in Watts(W).
(b) the average 1s integral of effective load and flywheel speed, over the total time period of each exercise bout, referred to as mean power output (MPO), expressed in Watts.
(c) the final 1s integral of effective load and flywheel speed, referred to as end power output (EPO), expressed in Watts.
(d) the time taken to achieve PPO.

A typical power output profile generated during a single 6s sprint by one of the subjects is shown in Figure 3.4.

Standardised Testing Procedure

All subjects who participated in the experiments had been previously familiarised with sprint-cycling until fully confident of producing an all-out effort from a stationary start. The familiarisation period spanned over two weeks and consisted of at least seven practice sessions, the last being four days prior to testing. During this period subjects also completed the whole exercise test and were thus, familiar with all experimental procedures. Subjects were also instructed to refrain from any form of intense physical exercise the day prior to testing.

On the day of the experiment, subjects reported to the laboratory following a fast of at least four hours and they were requested to sit on an examination couch in order to obtain resting blood and muscle samples. The subjects then underwent a period of low intensity exercise which consisted of stretching exercises and submaximal cycling for five minutes at 50 rpm against a resistance of 0.5Kg. This was followed by a standardised warm-up procedure which involved two 30s periods of submaximal cycling at 85 and 115 rpm against a resistance of 1.5Kg. These bouts of cycling were separated by 30s of recovery and served to re-accustom subjects to experimental procedures. This warm-up has previously been shown to result in only minor metabolic disturbances (Wootton, 1984). Following five minutes of stretching after this standardised warm-up, subjects performed the exercise test from a stationary start. Subjects were instructed to remain seated during all sprints. The optional saddle height had been determined previously for each subject and was adjusted so that the knee was still slightly flexed when the pedal was at the bottom of its travel. The feet were also secured to the pedals using toe-clips. Each subject was counted down into the next sprint which commenced on the command 3-2-1 GO! During the subsequent 30s recovery period subjects simply remained seated quietly on the cycle ergometer so that recovery period was standardised.

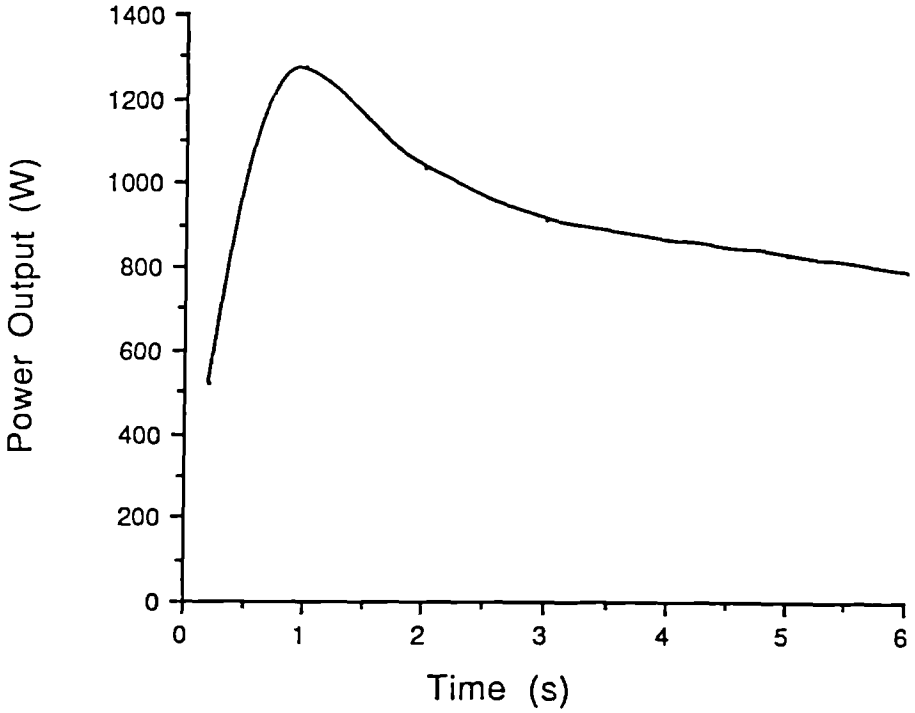


Figure 3.4. Power output profile (W) generated during a single 6s sprint (n=1).

Each sprint was preceded by the same instructions and was initiated from the same starting pedal position. Logging of data by the computer was initiated 2s before the subject was instructed to begin sprinting, in order to avoid timing errors that might have arisen had the subject predicted the start and starting slightly earlier. All subjects were highly motivated and were requested to cycle maximally from the start of the test and were verbally encouraged throughout. After completion of the exercise test subjects recovered for at least 15 minutes on the couch which was placed adjacent to the cycle ergometer.

Determination of Maximum Oxygen Uptake (VO_2 max)

During a preliminary session the subjects completed a VO_2 max test on a cycle ergometer (Monark Model 864). This test was conducted according to the procedures routinely used in this Department for both class experiments and for research projects (a generic protocol). The starting work-load chosen was high enough to ensure that exhaustion would occur within 6-12 min, the load being increased by 0.5Kg at the end of 3 minutes. Expired air samples were collected during the final minute of each load increment and the last 60s of exercise. The subjects breathed into a low resistance respiratory valve which was connected to a 150 litre Douglas bag by lightweight smooth bore tubing (Falkonia). Expired air samples were analysed for percentages of oxygen and carbon dioxide using a respiratory mass spectrometer (Centronics Ltd, model MGA200). The spectrometer was calibrated before each series of analyses with calibration gas and room air. The volume of expired air was determined by evacuating each Douglas bag through a dry-gas meter (Parkinson-Cowan Ltd) with an electrically operated vacuum pump. The temperature of the gas was determined by an electronic thermometer (Edale Instruments Ltd, Model C). At the time of each analysis prevailing room temperature and pressure were recorded and all volumes were later converted to STPD.

Heart Rate Monitoring

Heart rate was continuously monitored (Rigel, Cardiac Monitor 302) from 3 electrodes (Red dot, silver chloride) during all exercise tests and was at all times visible to the experimentor. A representative sample of the variation in heart rate responses during multiple-sprint cycling is illustrated in Figure 3.5.

3.3 COLLECTION AND ANALYSIS OF BLOOD AND MUSCLE SAMPLES

3.3.1 Blood Sampling and Analytical Procedures

Sample Collection

Venous blood samples were taken from an antecubital vein using an intravenous cannula (16 gauge) fitted with an injection diaphragm. The cannula was inserted under local anaesthetic (0.5ml of 1% lignocaine) and patency was maintained by displacing the blood contained within the cannula with isotonic saline containing 100 units.ml^{-1} of herapin. Blood samples were drawn at rest with the subjects in a recumbent position and at predetermined times during the exercise test and the recovery period following completion of the last sprint when the subjects returned to a recumbent position. The volume of blood taken for each sample in the above procedure was 10ml and was placed into calcium-heparinised tubes (250 units per tube) to prevent coagulation.

Sample Treatment and Analysis

Blood pH was determined immediately following collection on a pH/blood-gas analyser (Radiometer, model BMS3 Mk2). The percentage change in plasma volume was estimated from the resting and immediately post-exercise haematocrit and haemoglobin values (Dill and Costill, 1974). Haemoglobin concentration was determined photometrically using a standard cyanomethemoglobin method (Boehringer Mannheim GmbH, test-combination). In addition 20µl of blood was collected and immediately deproteinised in 200µl of 2.5% perchloric acid ($HClO_4$). The precipitate was separated by centrifugation (Eppendorf, model 5412). These samples were then frozen at -70°C and analysed fluorimetrically (Locarte, model 8-9) for blood lactate concentration (Maughan, 1982) and photometrically

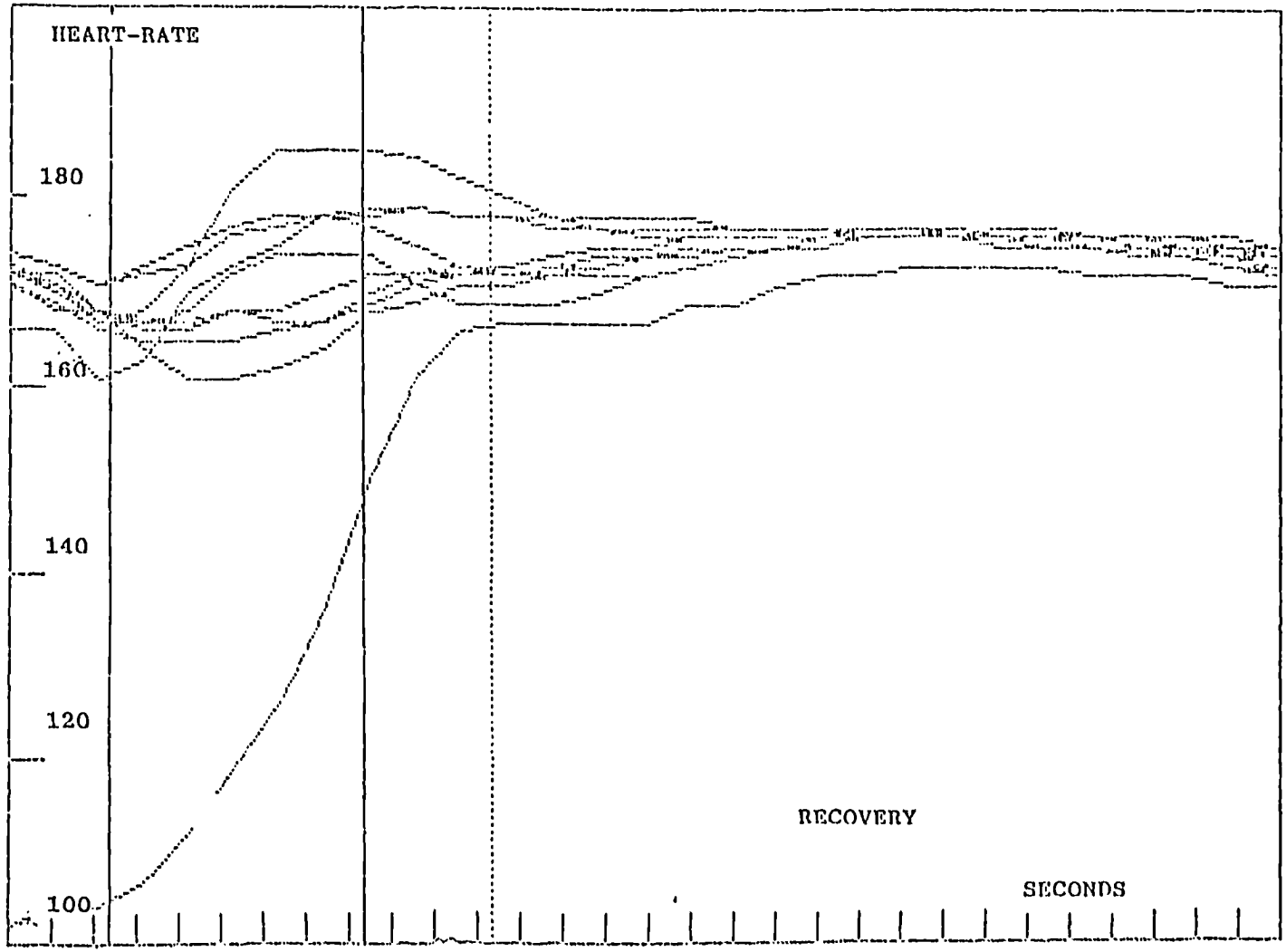


Figure 3.5. Heart rate response (beats/min) over the ten 6s sprints (n=1).

for blood glucose (Werner, Ray and Wielinger, 1970). The remaining blood (8-9ml) was centrifuged (at 6000 rpm) for 15 minutes at 3°C (Koolspin, model uP) after which duplicate 30µl of plasma were taken and stored at -70°C for the determination of sodium and potassium concentrations by flame photometry (Corning, model 435). The remainder was treated with 200µl of an antioxidising and chelating agent which was made up of 100mmol.l^{-1} EGTA (ethylenglycerol tetra-acetic acid) and 100mmol.l^{-1} GSH (reduced glutathione). The treated plasma was stored at -25°C and analysed at a later date for adrenaline and nor-adrenaline using high-performance liquid chromatography (Gilson model 302) with electrochemical detection (Davies, Kissinger and Shoup, 1981). In Study 3 (Chapter 6) plasma ammonia analysis was also performed, using a commercially available kit (Boehringer Mannheim GmbH - enzymatic UV method), within two days of collection, on duplicate 200µl aliquots which were placed in screw top plastic tubes and stored immediately in liquid nitrogen after blood centrifugation. Detailed assay procedures for the determination of blood lactate and plasma catecholamine concentrations are reported in Appendix A and the coefficient of variation for all the assays is shown in Table 3.1.

3.3.2 Muscle sampling and analytical procedures

Sample Collection

Muscle-biopsy samples were taken under local anaesthetic (2ml, 1% lignocaine), at rest prior to warm-up, at predetermined times during the exercise test and immediately after completion of the final exercise bout. The biopsies were obtained by a percutaneous needle biopsy technique (Bergström, 1962) with suction being applied. The sampling site selected for the muscle biopsies was the lateral portion of the quadriceps muscle (vastus lateralis). This muscle was selected because it is easily accessible with no risks and has been shown to be heavily involved when performing knee extensions during maximal cycling (Bigland-Ritchie and Woods, 1984). Muscle biopsies were taken within 3s from cessation of the sprints and were immersed in liquid nitrogen within 2s of sampling. Muscle samples of approximately 30-100mg wet weight were obtained.

TABLE 3.1 Coefficient of variation (CV%) for blood and plasma metabolite assays.

METABOLITE	CV% = $\frac{S.D.}{Mean}$ x 100
Blood lactate	1.6
Blood glucose	1.4
Plasma ammonia	2.9
Plasma potassium	1.1
Plasma sodium	1.1
Plasma adrenaline	6.9
Plasma noradrenaline	4.3

Sample Treatment

Muscle samples were removed from the biopsy-needle under liquid nitrogen and split into two pieces. A portion of the sample was freeze-dried and stored at -70°C for the determination of muscle metabolites whilst the remainder was placed in sealed glass tubes and stored in liquid nitrogen for the determination of muscle pH and buffering capacity (Study 2/Chapter 5).

Freeze-dried muscle was dissected free of visible blood and connective tissue and powdered using an agate pestle and mortar. The powdered sample was placed in plastic tubes and washed twice with 1ml of petroleum ether (30-40°C), to remove the fat. The ether has the additional benefit of allowing the removal of any blood mixed with the powder, as blood remains in suspension much longer than the particles of muscle after mixing, thus allowing it to be discarded with the ether. Each time the sample was thoroughly mixed and centrifuged, removing as much ether as possible. Finally, the tube was left open for the remaining of the ether to evaporate until samples were dried. Samples were then weighed on an electrical balance accurate to 1ug and were stored at -70°C until further analysis.

Muscle Metabolite Assays

Extraction Procedure: On the day of analysis, muscle metabolites were extracted by adding an ice-cold solution containing 0.5mol.l^{-1} $HClO_4$ and 1mmol.l^{-1} EDTA.Na_2 (ethylenediamine tetra-acetic sodium salt) onto the freeze-dried muscle powder kept in liquid nitrogen, so that $HClO_4$ froze on introduction into the tube. For every 12.5mg of powder, 1ml $HClO_4$ was added (Harris et al, 1974). Each sample was immediately placed in the freezer at -25°C for $HClO_4$ to thaw and then on ice when all samples were ready for agitation. Approximately 30 samples were agitated together in a Stuart flask shaker (speed 10) for 15 minutes at 0°C. This was followed by centrifugation (6000 rpm) for 10 minutes at 0°C. The supernatant was then removed, carefully measured and subsequently neutralised by the addition of one-fourth volume of 2.1mol.l^{-1} $KHCO_3$ (potassium-bicarbonate). Extracts were then thoroughly mixed and left on ice uncapped for 15 minutes to let the formed CO_2 to evolve. Finally, samples were recentrifuged for 5 minutes at 0°C and the supernatant was

removed. The pH of the extract was 7.0 and checked before analysis was commenced. Following this procedure 1ml of the extract was equivalent to 10mg of muscle powder (Harris et al, 1974).

Analytical Procedures: All assays for each muscle extract were performed over a period of three days. Immediately after sufficient sample, for the assay being carried out, had been removed the muscle extract was kept on ice and then stored at -70^{o}C overnight. Repeated freeze-thaw cycles of the standard solutions has been shown not to lead to significant loss of metabolites when using this method. Batches of approximately 30 samples were assayed at each time. The analysis of PCr, Cr and adenine nucleotides (i.e. ATP, ADP and AMP) was carried out on the day of extraction because of the lability of these compounds. The hexose-monophosphates, glucose, F-1,6-diP and triose phosphates were assayed on the following day and lactate, pyruvate and glycogen on the third day. Glycogen was determined both on the neutralised extract (acid-soluble glycogen) and also on the precipitated muscle pellet, after the extraction procedure (acid-insoluble glycogen) by prior hydrolysis in 1mol.l^{-1} HCl (hydrochloric acid).

Assays were developed using resting human quadriceps femoris muscle and where necessary, modifications of previously described methods were introduced (Lowry and Passonneau, 1972; Harris et al, 1974). The determination of all muscle metabolites involved fluorimetric analyses (Locarte, model 8-9) based on enzyme catalysed reactions, the coenzymes NAD^{+} and $NADP^{+}$ being simultaneously reduced to NADH and NADPH, respectively. Acid insoluble glycogen was assayed photometrically (Eppendorf, model 1101M) using a commercially available glucose kit (Boehringer-Marmheim GmbH - Glucose test combination, GOD/Period method). Detailed assay procedures are reported in Appendix B. Whenever possible samples were assayed in duplicate except where a small muscle sample precluded this. It was possible, however, to determine the concentrations of all the metabolites on as little as 2mg of freeze-dried muscle powder.

Buffers, co-factors (Grade-I) and enzymes were obtained as standard commercial items from Boehringer and Sigma. All reagents had been previously made up with double distilled water and stored at 4°C. Enzymes were added just prior to use in amounts sufficient to give a linear end point within 20-30 minutes. Standards were freshly prepared prior to each assay by weight using Grade-I chemicals. The standard curves were always found to be linear (r = 0.999) and metabolite concentrations were calculated using the resulting standard regression equation taking into account double-distilled water blanks, extract blanks, standards and dilutions. The coefficient of variation for the assays is shown in Table 3.2.

The reference base for muscle metabolite concentrations is dry weight to avoid changes in concentration due to water shift during exercise (Bergström et al, 1971; Sahlin et al; 1978). In addition, the content of energy-rich phosphagens and metabolites has been adjusted to the highest content of total creatine (PCr + Cr) in each subject series as follows:

$$\frac{\text{metabolite concentration}}{\text{total (PCr + Cr)}} \times \text{total highest (PCr + Cr)}$$

(Harris et al, 1976)

By these means it was thought to compensate for admixture of connective tissue and other non-muscular elements (i.e. fat) and blood contamination in the muscle sample. However, muscle lactate and glucose were not adjusted for total creatine content as these metabolites occur in significant amounts in both muscle and blood and the amount present of the contaminating blood of the samples was not possible to determine.

Muscle pH Determination

Methodological Considerations: The homogenate technique for estimating the pH of human skeletal muscle has been widely used in exercise-related research, mainly because of its simplicity. However, muscle pH measured by this technique is limited because it represents a mixture of both extracellular and intracellular compartments and thus may be influenced

TABLE 3.2 Coefficient of variation (CV%) for muscle metabolite assays

METABOLITE	CV% = $\frac{S.D.}{Mean}$ x 100
Glycogen:	
Acid soluble	3.7
Acid insoluble	1.3
Adenosine triphosphate	3.0
Adenosine diphosphate	1.0
Adenosine monophosphate	1.6
Phosphocreatine	1.8
Creatine	2.4
Glucose	3.8
Glucose-1-phosphate	4.5
Glucose-6-phosphate	3.1
Fructose-6-phosphate	3.6
Fructose-1,6-diphosphate	3.6
Triose phosphates	2.9
Lactate	1.4
Pyruvate	3.6

by the volume, buffering capacity and pH of the extra- and intracellular fluids (Hultman and Sahlin, 1980). It has been estimated that the extracellular fluid in the homogenate prepared *from wet muscle may* increase the measured pH by about 0.03pH units in samples taken at rest whereas, following exercise, possible contamination of samples by excessive amount of blood could increase this even further (Sahlin, 1978). It has also been reported that during homogenisation and measurement of pH, gradual loss of CO_2 from the sample could lead to a gradual increase in pH (Costill, Sharp, Fink and Katz, 1982). However, it was later shown that a 10 to 12-fold dilution of the muscle sample with homogenising solution, drastically reduced the PCO_2 gradient from the sample to the environment. Together with homogenisation at 0^oC and use of tightly sealed tubes for homogenisation, most of the CO_2 (40-60%) remained in the sample following a 10-minute incubation (Spriet, Söderlund, Thomson and Hultman, 1986). Furthermore, it has been shown that during muscle pH measurements at 37^oC, despite significant ATP and PCr hydrolysis occurring (60% and 35%, respectively) within 30s, there was no significant effect on the pH of muscle homogenates obtained at rest or after electrical stimulation (Spriet et al, 1986). The volume of homogenising solution added to the muscle samples has also been varied (Costill et al, 1982; Spriet et al, 1986; Cheetham 1987). Recently, Cheetham (1987) examined the effect of different dilutions of samples obtained from rat muscle on pH and buffering capacity. It was shown in that study that although muscle pH was almost unaffected by dilution, selected in the range of 10-50µl of homogenising solution per mg wet muscle, muscle buffering capacity determined by titration was affected. The highest buffer values being obtained at the weakest dilution (100.6 $\pm$ 11.7 slykes). In this thesis a dilution of $25\mu l.mg^{-1}$ wet muscle was selected because it could provide sufficient volume of homogenate to determine pH and buffering capacity on samples as small as 5mg.

Analytical Procedures: Muscle pH was determined on muscle homogenates as previously described (Marlin, 1989). Wet muscle was removed from storage, placed in a disposable plastic cuvette of predetermined weight and weighed out frozen (range 14.7-47.2mg) on an electrical balance accurate to 1µg, within 7-10 seconds of exposure to laboratory

conditions. The sample was immediately returned to liquid nitrogen and the volume of the homogenising solution, maintained at $0^{o}C$ was determined, the ratio being $25\mu l.mg^{-1}$ wet muscle (wm). The homogenising solution was previously made up and contained $145mmol.l^{-1}$ KCl, $10mmol.l^{-1}$ NaCl and $5mmol.l^{-1}$ iodoacetic acid (sodium salt). This iodoacetic acid was included to block glycolysis which would otherwise occur on homogenisation (Sahlin, 1978). The required volume of this solution was added to the cuvette containing the sample, upon removal from liquid nitrogen, and was immediately homogenised for 1 minute with a Polytron Homogeniser at speed 7. It was always made certain that significant amounts of homogenate did not remain attached to the shaft of the homogeniser. Homogenates were equilibrated to $37^{o}C$ for 5 minutes and pH readings were taken every 30 seconds during this period, using a MI-410 microelectrode (Microelectrode Inc) fitted to a Corning 150 pH/ion meter (Figure 3.6). The electrode was calibrated each time prior to homogenisation and the initial response after immersion into the homogenised medium was 5 seconds. Muscle pH was calculated as the average value over the 5 minute equilibration period. The coefficient of variation was determined on 10 samples of approximately 20-35mg wet muscle each obtained from freeze clamped resting rat muscle and was calculated to be 0.51%.

Determination of Muscle Buffering Capacity

Methodological Considerations: The muscle buffer value (B) has usually been determined in the past either by fixed acid titration of a homogenate (in vitro) or by measuring the change in lactic acid concentrations ($\triangle$[LA]) and muscle pH ($\triangle$pH) following high-intensity exercise to fatigue (in vivo, $B = \frac{\triangle[LA]}{\triangle pH}$)

The former is considered to measure physicochemical buffering only, whereas the latter also includes H^{+} uptake by metabolic processes and possibly the unequal efflux of H^{+} and lactate from muscle, thus resulting in higher values when compared to that estimated by titration (Hultman and Sahlin, 1980). Titration of muscle homogenates prepared from wet muscle obtained from humans (Cheetham, 1987) and horse (Marlin, 1989) have indicated that the shape of the titration curve is non-linear

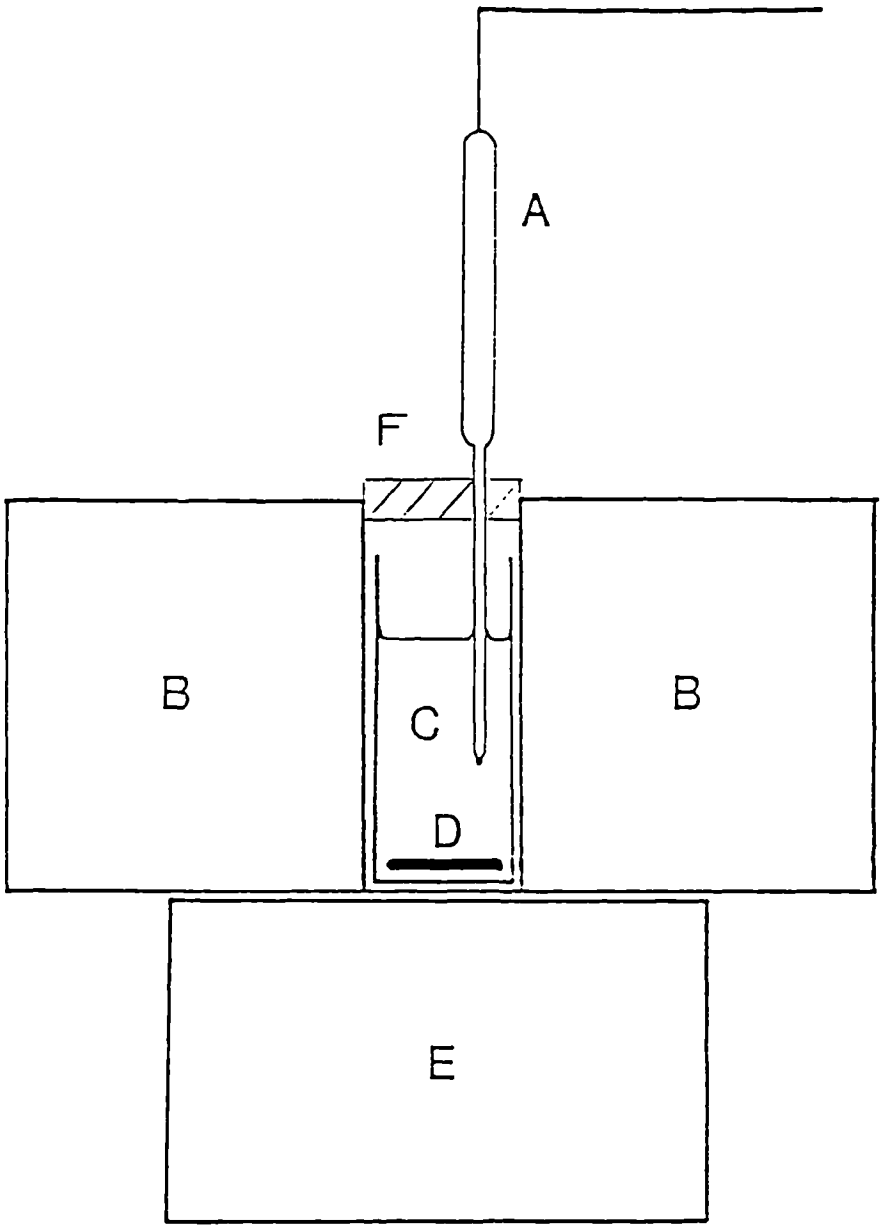


Figure 3.6. Apparatus for determination of muscle buffering capacity. A-Electrode; B-Waterbath; C-Stirring bath; E-Magnetic stirrer; F-Lid (after Marlin, 1989).

over the pH range 7.0 to 6.0 and instead two linear falls in pH have been observed with a different slope. The pH at which the slope changed, varied from 6.9 to 6.7. Because of this non-linear response to H^+, estimates of buffering capacity are effected by the pH range selected for titration (Marlin, 1989). The pH of resting human muscle is 7.0-7.1 and the pH frequently observed following intense exhaustive dynamic exercise is 6.5 (see section 2.2.). It was thought therefore, as previously suggested (Marlin, 1989), that in this thesis, muscle buffering capacity determined by titration should be expressed as the µmol H^+ required to change the pH of 1g of wet muscle from pH 7.1 to 6.5.

Analytical Procedures: Muscle buffering capacity was determined by titration on the muscle homogenate after the pH determination was made, as previously described (Marlin, 1989). Homogenates were titrated to pH 6.5 against 5µl aliquots of a standard 10mmol.l^{-1} solution of HCl added with a 25µl Hamilton positive displacement pipette (Hamilton). If necessary, the pH was adjusted upwards to 7.1 with 50mmol.l^{-1} NaOH to give a common starting point. For verification purposes the sample was titrated back to 7.1 using 5µl aliquots of 10mmol.l^{-1} solution of NaOH. Both HCl and NaOH (10mmol.l^{-1}) solutions were made up from commercially available volumetric solutions (BDH). Homogenates were stirred continuously and maintained at 37°C throughout the titration period (approximately 4-7 minutes). It has been shown that the effect of different incubation times lasting up to 60 minutes on buffering capacity by titration is negligible (Marlin, 1989). Muscle buffering capacity (B) was expressed in µmol.g^{-1} wm (over pH range 7.1-6.5) and was calculated as follows:

$$B = \frac{\mu l \text{ HCl (or NaOH) added} \times \text{titration standard concentration}}{\text{weight of muscle sample(g)}} \quad (\text{mmol}.l^{-1})$$

To accurately determine the volume (µl) of HCl or NaOH (10mmol.l^{-1}) added over pH range 7.1-6.5 the titration curve of each homogenate was drawn as shown in Figure 3.7.

The coefficient of variation on 10 muscle homogenates from the same rat muscle prepared as described above was found to be 3.9% for the total procedure (i.e. from division of muscle to titration inclusive).

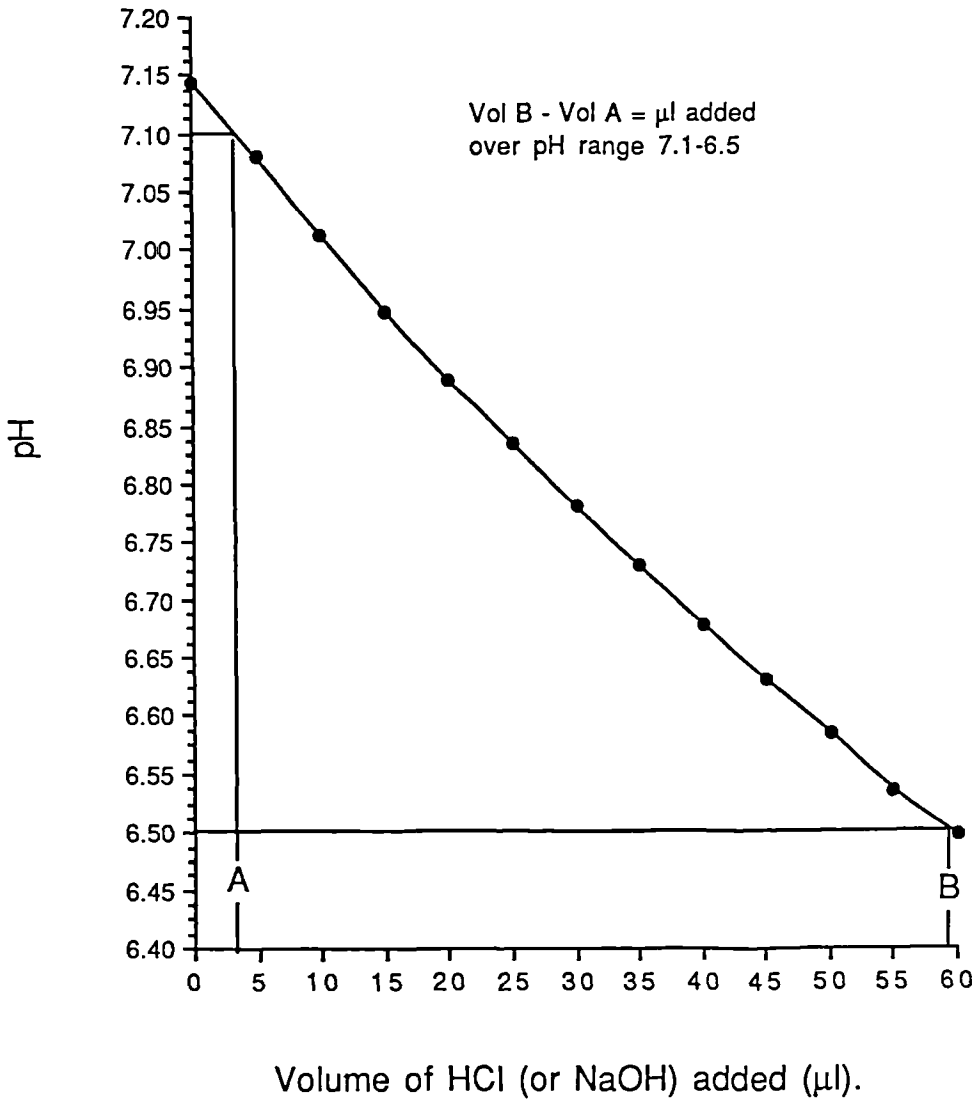


Figure 3.7. Determination of volume (µl) of HCl or NaOH added for titration of muscle homogenate.

4. HUMAN MUSCLE METABOLISM DURING INTERMITTENT BRIEF MAXIMAL EXERCISE

4.1 INTRODUCTION

In the past, it was proposed that the energy requirements during the first 10-15s of dynamic exercise leading to exhaustion in about 30-40s could be entirely met by the intramuscular stores of ATP and PCr (Margaria et al, 1964). It was also suggested that intermittent intense exercise could be carried out indefinitely if the exercise intensity and duration were such that the energy expenditure during each bout did not exceed that corresponding to the alactacid fraction of the O_2-debt, and if the rest intervals were long enough to allow the complete repayment of the O_2-debt (Margaria et al, 1969). These suggestions however, were based on blood lactate and oxygen consumption measurements. Further studies on intermittent intense exercise using brief work periods (10-15s), interrupted by short rest intervals (15-20s), have demonstrated that the contribution of anaerobic glycolysis to the total energy demand was either of minor importance (Saltin and Essén, 1971) or considerably less compared with that if work of a similar intensity was performed continuously (Essén et al, 1977; Essén and Kaijser, 1978). These findings suggest a muscle oxygen store (such as myoglobin) which could be rapidly reloaded during the recovery intervals, thereby allowing a greater aerobic metabolism (Saltin and Essén, 1971; Essén et al, 1976). A greater lipid contribution to energy metabolism during intermittent rather than continuous intense exercise, at the same work load, was also suggested (Essén and Kaijser, 1978).

However, the former studies utilised exercise intensities that cannot be described as maximal. It is now accepted that glycogenolytic processes leading to lactate formation occur almost immediately with the onset of maximal dynamic exercise (Boobis et al, 1982; Jacobs et al, 1983b). Moreover, performance during maximal intermittent exercise has been found to be dependent upon both the preceding number of exercise bouts and the recovery duration. Wootton and Williams (1983) have demonstrated more pronounced fatigue and higher blood lactate concentrations when five bouts of 6s maximal exercise were performed with 30s intervals compared with those observed when the recovery was

60s. It was postulated that the fatigue associated after only 30s recovery was the result of incomplete replenishment of the PCr stores. Therefore, a greater demand was placed on glycolysis to maintain ATP concentrations during subsequent bouts of exercise. Thus, inadequate PCr recovery may not be a limiting factor per se, but rather the acidosis resulting from increased glycolysis may be the main cause of fatigue.

The aim of the present study was to describe the metabolic changes in muscle and blood of subjects performing intermittent exercise of maximal intensity in order to examine the relationship between muscle metabolism and fatigue. The exercise protocol consisted of ten 6s sprints on a cycle ergometer with 30s recovery between each sprint. During this type of exercise fatigue is defined as a gradual decrement in power output during successive exercise periods.

4.2 METHOD

Subjects

Eight healthy male physical education students whose mean (± s.d.) age, height and weight were 26.7 ± 8.4 years, 117.6 ± 11.4cm and 71.8 ± 11.4Kg, respectively, gave their informed consent and volunteered to take part in this study, which was approved by the University Ethical Committee.

Protocol

All subjects had been previously familiarised with maximal cycling. At least four hours elapsed between the subjects' last meal and the start of the experiment following the warm-up procedure (see Chapter 3). The exercise-test consisted of ten maximal 6s sprints on the instrumented cycle ergometer with 30s rest intervals between each sprint (Figure 4.1). Heart rate was monitored continuously during the exercise-test and for the first 10min of a resting recovery subsequent to the exercise test. The subjects were transferred from the cycle ergometer to a recumbent position.

Maximal oxygen uptake was determined on the cycle erogmeter on a separate session.

Blood and muscle sampling

Venous blood samples were taken at rest, immediately after the first, fifth, ninth and tenth sprints and at 3min, 5min and 10min following completion of the last sprint. The blood samples were analysed for blood pH, lactate, glucose and plasma catecholamines, sodium and potassium concentrations. Changes in plasma volume were estimated from the immediate post-exercise haematocrit and haemoglobin concentrations.

Muscle samples were taken from the vastus lateralis muscle of the same leg at rest prior to warm-up and immediately after the first sprint and from the other leg 10s prior to and immediately after the tenth sprint. The order of which leg was sampled first was randomly assigned. Muscle samples were stored in liquid nitrogen until they were freeze dried after which they were dissected free of visible blood and connective

tissue and powdered. Fat was removed by petroleum ether extraction. Acid extracts of muscle were prepared as previously described (see Chapter 3) and analysed for PCr, ATP, ADP, AMP, Cr, G-1-P, G-6-P, F-6-P, F-1,6-diP, triose phosphates, pyruvate, lactate and the acid soluble fraction of glycogen. The acid insoluble fraction of glycogen was also determined on the precipitated muscle pellet, after the extraction procedure.

Statistical Analysis

A one-way analysis of variance for correlated means was used to examine the differences in performance indices and metabolite concentrations over time and a Pearson product moment correlation was performed to examine the relationship between variables. Statistical significance was accepted at the 5% level and was established prior to the investigation. Results are presented as mean $\pm$ s.d.

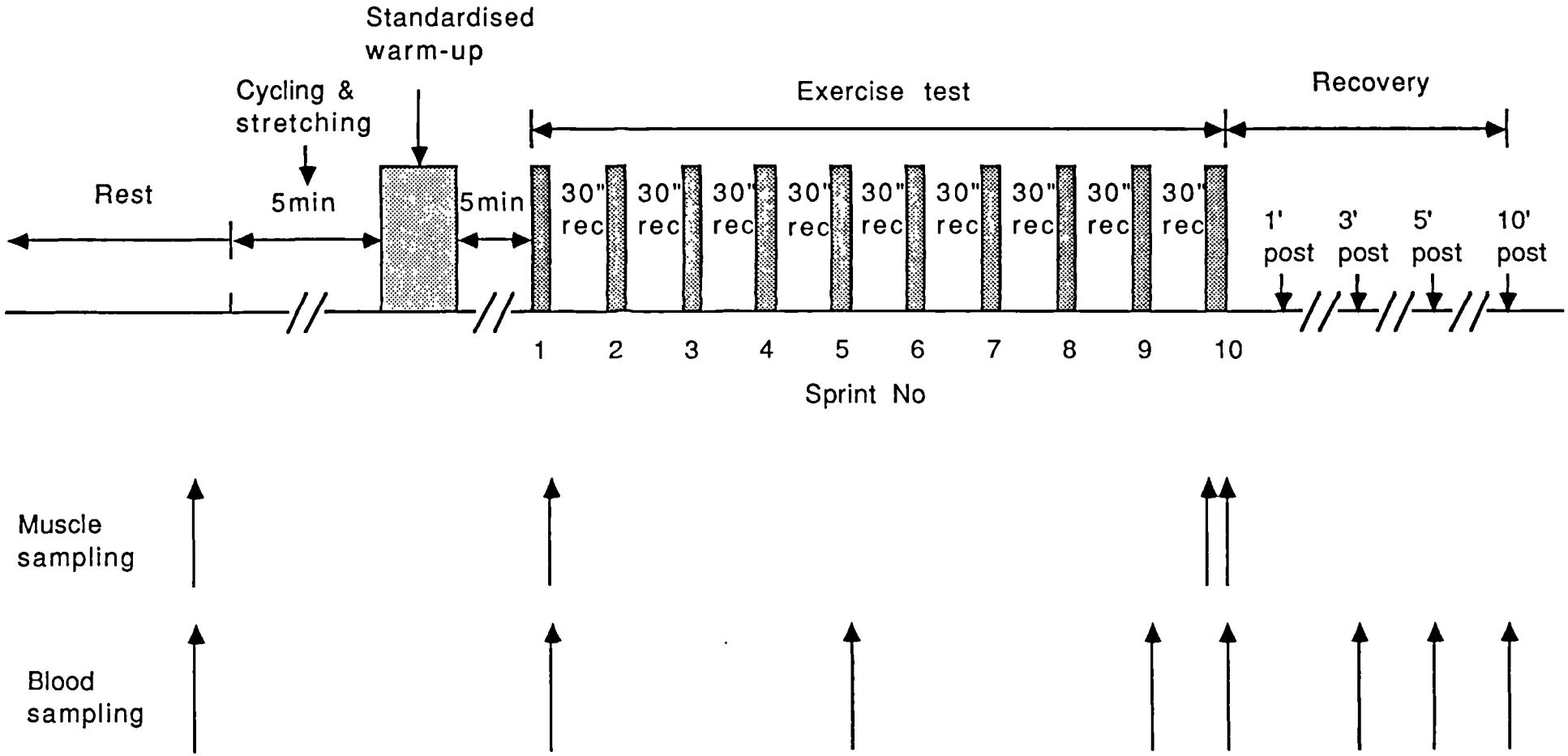


Figure 4.1. Schematic illustration of study's protocol.

4.3 RESULTS

Power Output

The mean power (MPO), peak power (PPO) and end power (EPO) values recorded during each of the ten 6s sprints are shown in Figures 4.2, 4.3 and 4.4, respectively. The PPO generated during the first sprint (1253.3 ± 334.8W) was approximately five times greater than that which elicited the subjects' VO_2 max (253.6 ± 57.8W). The highest mean power was reached in the first sprint (870.1 ± 159.2W) after which there was a decline in power output. At the fifth and final sprints MPO was decreased by 12.6% ($p<0.01$) and 26.6% ($p<0.01$) of the maximal value, respectively. It was calculated that 47.5% of the fall in MPO over the ten sprints occurred in the first five sprints. Moreover, the fall in MPO was significantly less ($p<0.01$) from sprints 4 through to 10, indicating that four 6s bouts of maximal cycling is short enough to elicit fatigue induced decrements in performance with 30s recovery. The decrease in PPO after five and ten sprints averaged 15.9% ($p<0.05$) and 33.4% ($p<0.01$), respectively, whereas the decline in EPO for the same sprints was 22% ($p<0.01$) and 31.1% ($p<0.01$), respectively.

To give an indication of performance over the first five sprints and the entire sprint-test, MPO values were averaged to produce an 'average MPO per sprint'. The average MPO per sprint over five and ten sprints were 817.8 ± 43.0W and 746.8 ± 82.9W ($p<0.01$). The respective average PPO values were 1155.5 ± 71.9W and 1040.8 ± 136.7W ($p<0.01$). It is also of interest to note that the amount of work completed over the first five sprints was 54.5% of the total work done over the entire sprint-test (44805 ± 7610J). In addition, the development of PPO generated during the early stages of each sprint did not change with successive bouts of exercise. The time taken to achieve PPO in all sprints was within the first 2s of exercise.

Blood lactate and blood pH

The first sprint resulted in a small increase in blood lactate concentration (Δlactate = 1.3mmol.l^{-1}) but after sprint 5, lactate concentration had increased 15-fold from 0.6 ± 0.1mmol.l^{-1} at rest to 9.2 ± 1.5mmol.l^{-1} ($p<0.01$). Following completion of sprint 9, blood

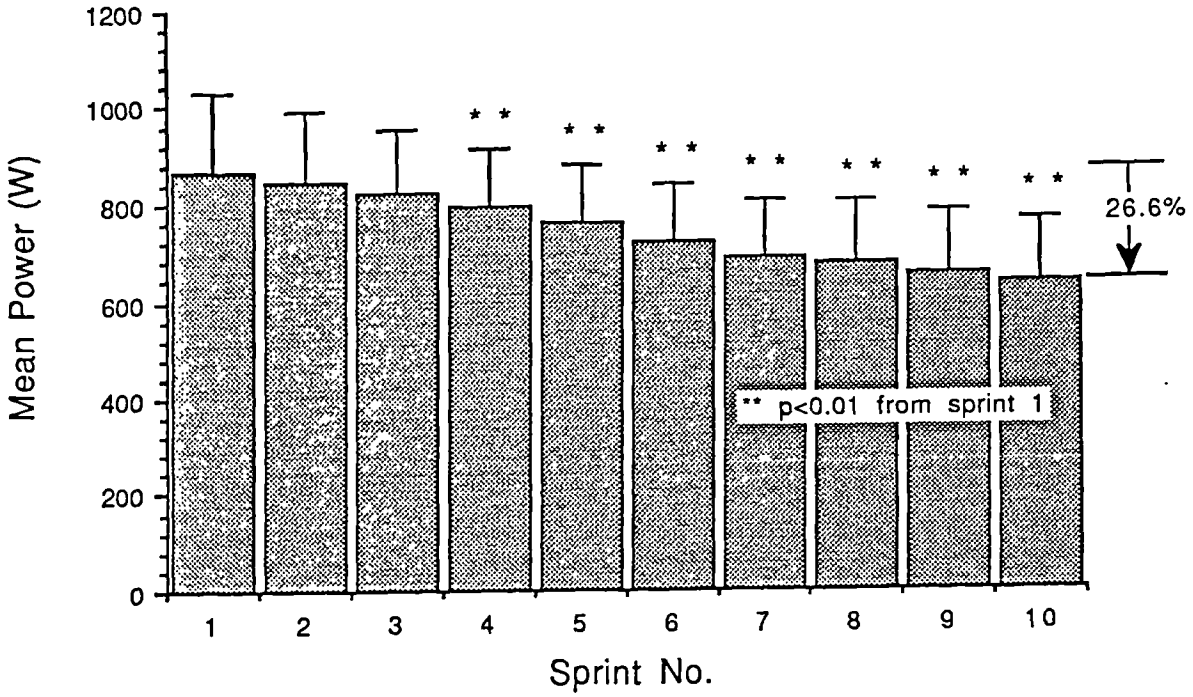


Figure 4.2. Mean power output (W) for each of the ten 6s sprints (mean ± s.d., n=7).

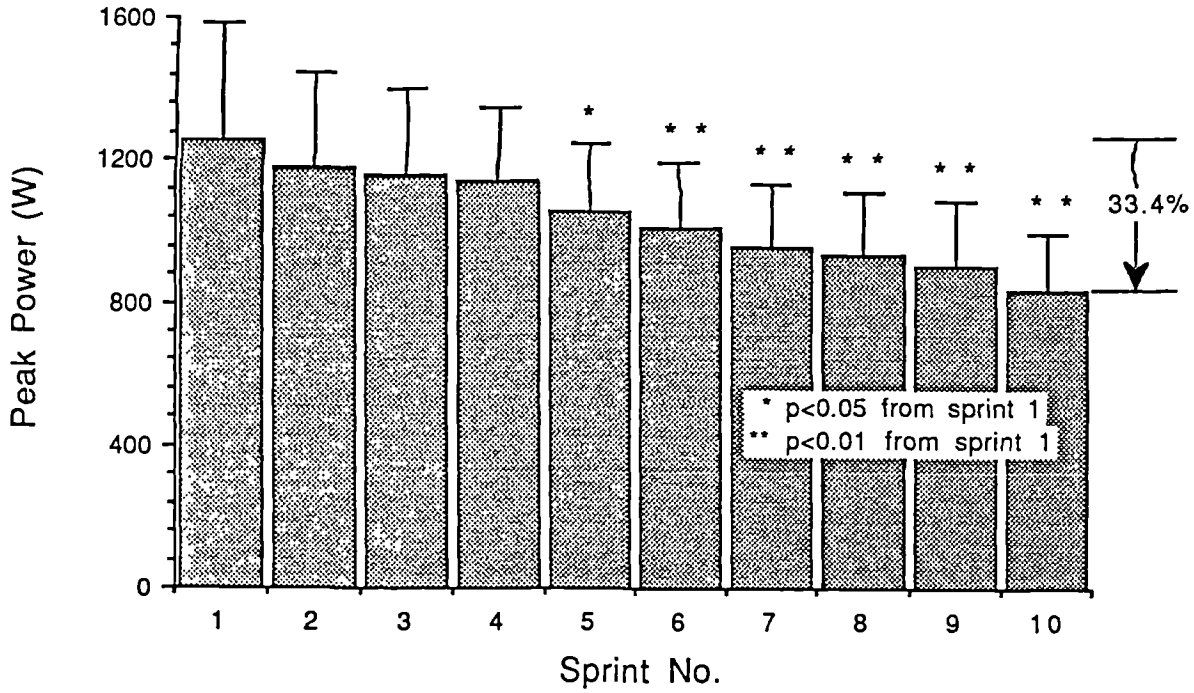


Figure 4.3. Peak power output (W) for each of the ten 6s sprints (mean ± s.d., n=7).

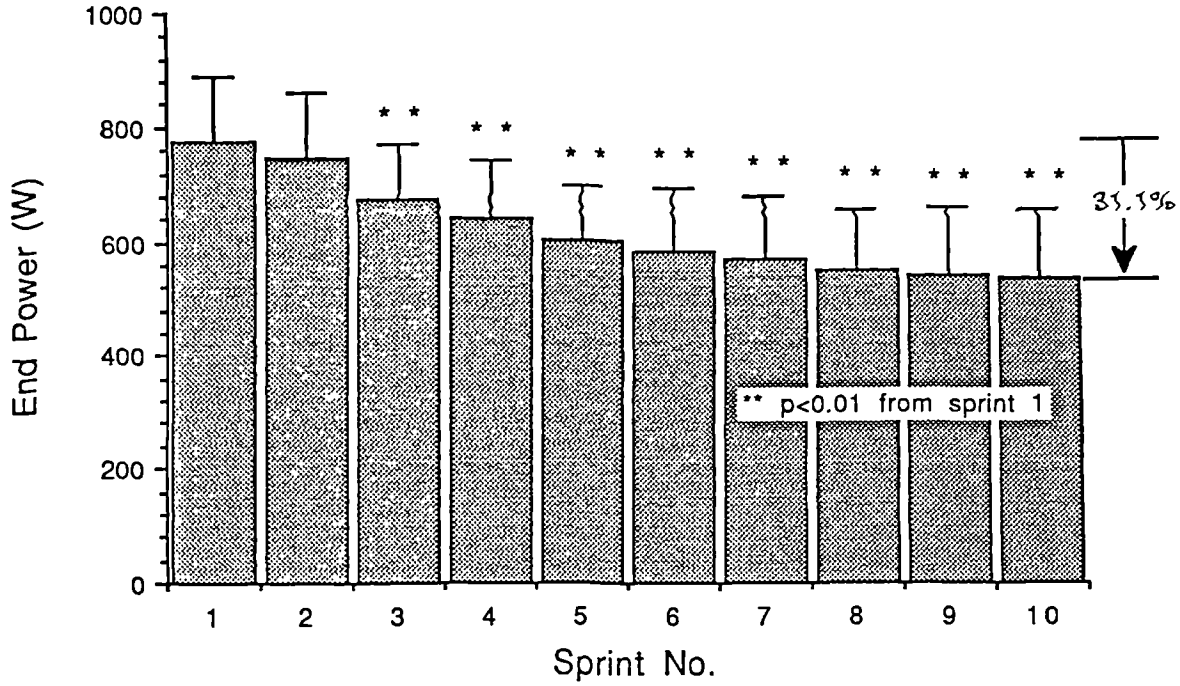


Figure 4.4. End power output (W) for each of the ten 6s sprints (mean ± s.d, n=7).

lactate had increased further to 12.6 ± 1.5mmol.l^{-1} ($p<0.01$) and did not change during the 10min of post-exercise recovery (Figure 4.5). A good correlation ($r=0.881$, $p<0.05$, $n=6$) was found between the increase in blood lactate at the end of sprint 5 and the total amount of work done over the first five sprints. The relationship for the second five sprints, during which 45.5% of the total work done was performed was not as strong. However, those individuals who showed the greatest fall in MPO after ten sprints tended to have the largest changes both in blood lactate at 5min post-exercise ($r=0.916$, $p<0.01$, $n=7$) and in blood pH immediately after sprint 10 ($r=0.820$, $p<0.05$, $n=6$) from resting values.

There was only a small change in blood pH by the end of the first sprint (ΔpH = 0.02). However, it decreased by 0.27 units (from 7.37 ± 0.03 at rest to 7.10 ± 0.03) by the end of the exercise test ($p<0.01$) and remained low for the first 3min of the passive recovery (Figure 4.6). During the subsequent 7min of recovery blood pH recovered to 7.16 ± 0.07 ($p<0.01$) indicating that the time-course of recovery during this period was different to that for blood lactate. The decrease in blood H^+ concentrations occurred at a faster rate than the decrease in lactate following exercise. Those subjects who exhibited the greatest change in pH immediately after exercise, from resting values, also produced the largest change in blood lactate concentration at 3min post-exercise ($r=0.944$, $p<0.01$, $n=6$).

Blood glucose

The responses of blood glucose to the exercise-test are shown in Figure 4.7. Blood glucose concentration increased from 3.9 ± 0.2mmol.l^{-1} at rest to 4.6 ± 0.5mmol.l^{-1} after nine sprints ($p<0.01$) and continued to rise until 5min into the recovery period (5.6 ± 0.6mmol.l^{-1}, $p<0.01$). The degree of hyperglycemia varied considerably between subjects and was maintained throughout the exercise and recovery period.

Plasma Catecholamines

The first sprint resulted in a 5-fold increase in plasma AD concentration from 0.4 ± 0.2nmol.l^{-1} at rest to 1.9 ± 1.0nmol.l^{-1} ($p<0.05$) and in a 2-fold increase in plasma NA from 1.7 ± 0.4nmol.l^{-1} at

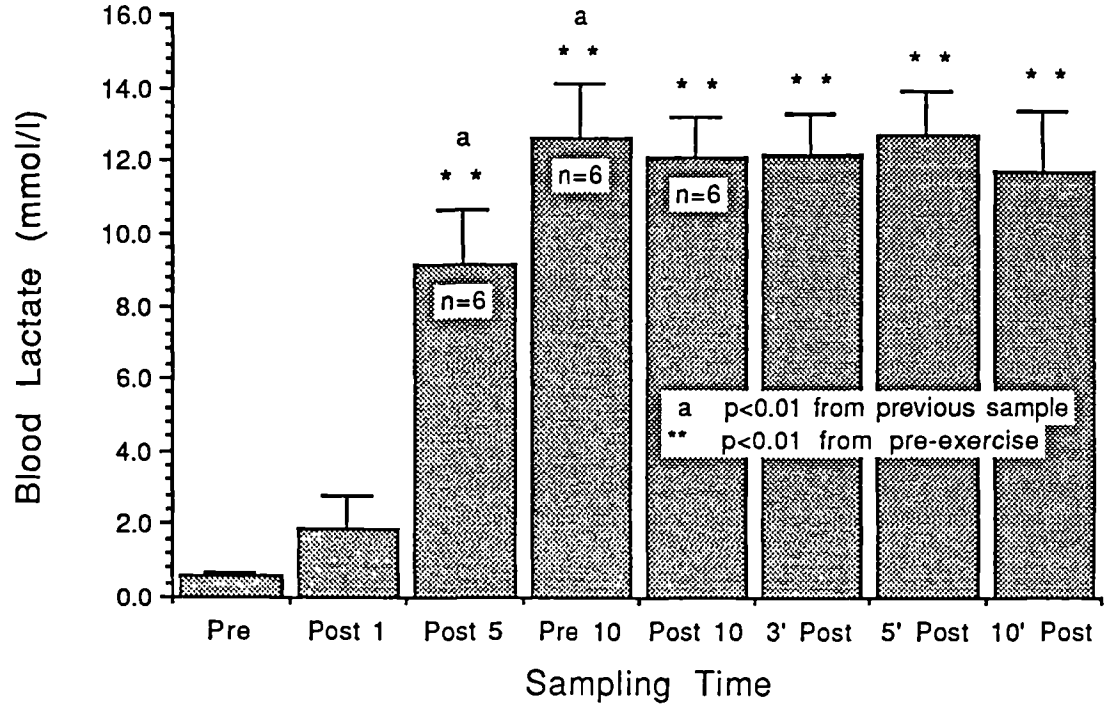


Figure 4.5. Blood lactate concentrations (mmol/l) during exercise and recovery (mean ± s.d., n=7).

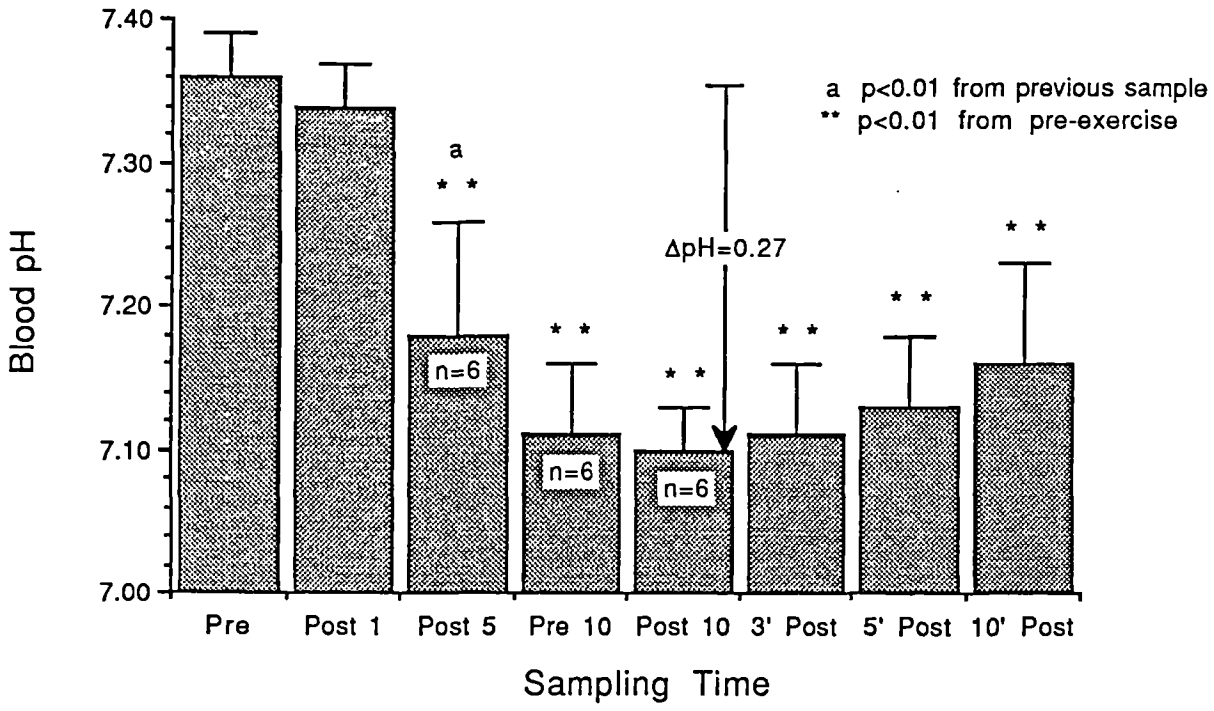


Figure 4.6. Blood pH during exercise and recovery (mean ± s.d., n=7).

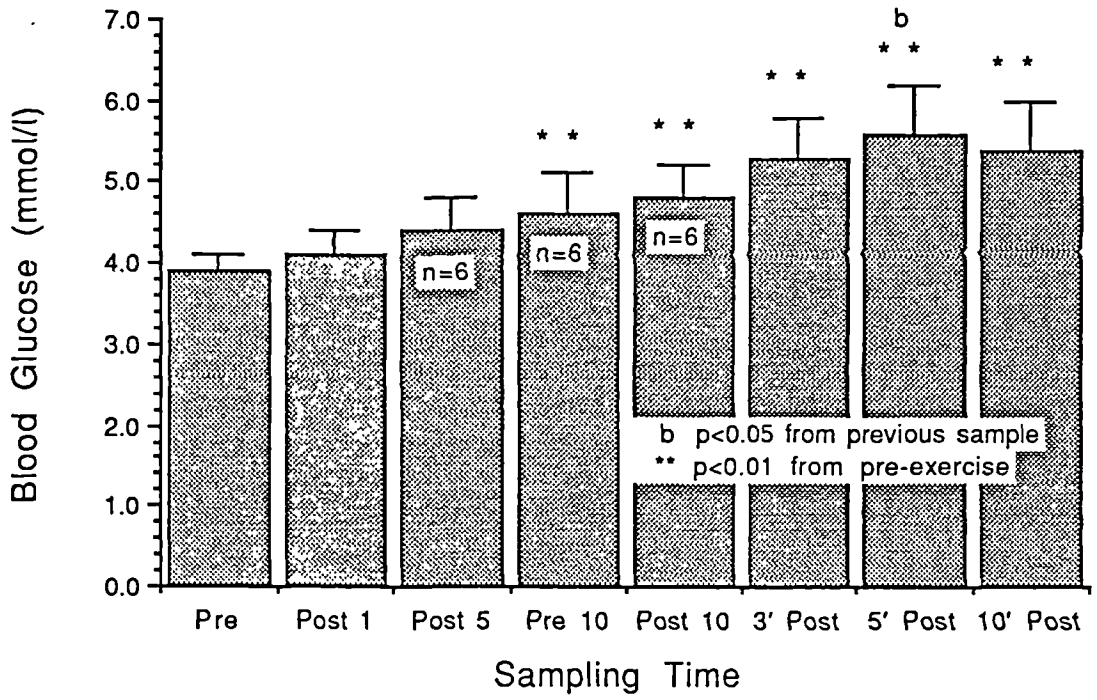


Figure 4.7. Blood glucose concentrations (mmol/l) during exercise and recovery (mean ± s.d., n=7).

rest to 3.3 ± 1.1nmol.l^{-1}. Both plasma AD and NA concentrations increased after sprint 5 to 4.2 ± 1.4nmol.l^{-1} ($p<0.01$) and 15.7 ± 2.7nmol.l^{-1} ($p<0.01$), respectively. The peak values occurred following completion of sprint 9, representing a 13-fold increase from resting values in AD to 5.1 ± 1.5nmol.l^{-1} ($p<0.01$) and a 13-fold increase in NA to 22.3 ± 6.5nmol.l^{-1} ($p<0.01$). The NA concentration remained over the pre-exercise values throughout the recovery period ($p<0.01$) (Figure 4.8) whilst AD concentrations were not different from resting after the immediate post-exercise sample (Figure 4.9).

Plasma Electrolytes

Plasma potassium concentration was elevated after sprint 1 from 3.2 ± 0.3mmol.l^{-1} at rest to 3.7 ± 0.4mmol.l^{-1} ($p<0.05$) but did not increase further in response to successive bouts of exercise (Figure 4.10). Following completion of the last sprint plasma potassium decreased by 24% of the pre-sprint 10 value ($p<0.05$). It continued to decrease until 3min post-exercise when the lowest potassium value (2.2 ± 0.3mmol.l^{-1}) occurred. During the subsequent 7min of recovery, plasma potassium concentration had recovered to 2.6 ± 0.3mmol.l^{-1}, but it was still different from resting values ($p<0.01$). A good correlation was found between blood pH and plasma potassium concentration after sprint 1 ($r=0.815$, $p<0.05$, $n=7$). Comparisons of plasma sodium concentrations at each time of sampling during exercise and recovery revealed no difference from resting values (Figure 4.11).

Change in plasma volume

The exercise-test resulted in an average decrease in plasma volume of 12.1 ± 4.5% ($p<0.01$) which is a marked change considering that subjects only exercised for a total of one minute. The changes in the concentrations of blood lactate and glucose, plasma catecholamines and electrolytes, in response to the exercise test, were several fold higher than those in plasma volume. Therefore, changes in blood and plasma metabolites cannot be explained by changes in plasma volume.

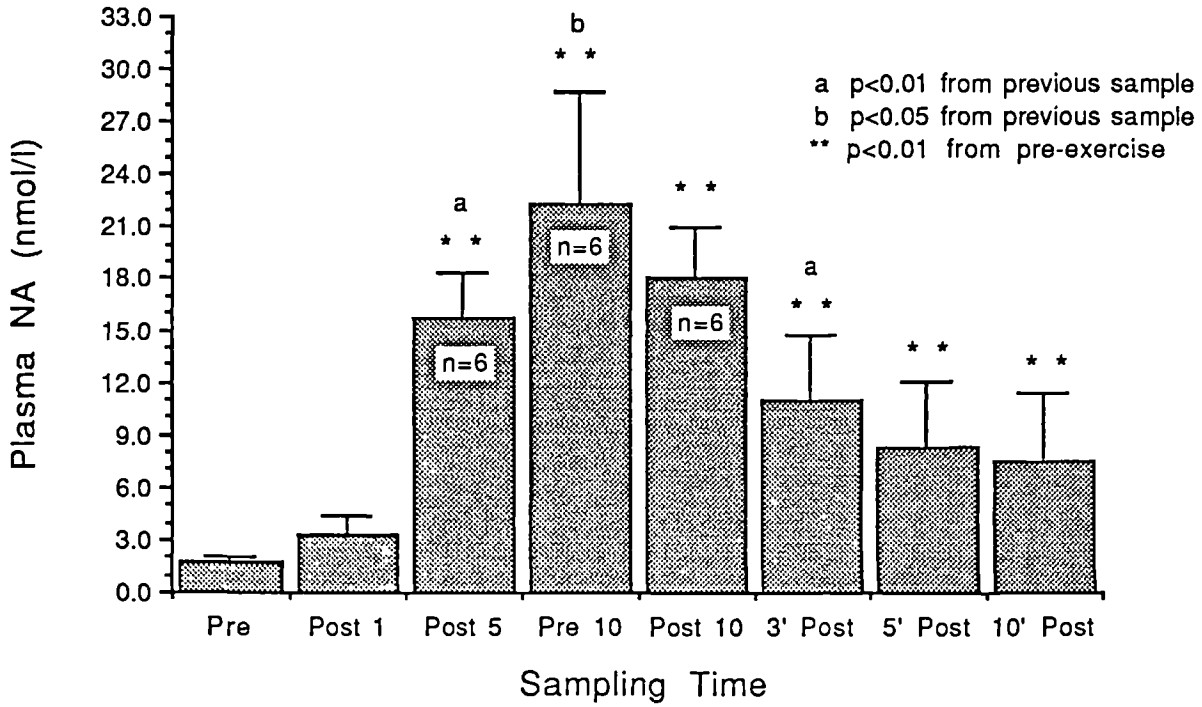


Figure 4.8. Plasma nor-adrenaline concentrations (nmol/l) during exercise and recovery (mean ± s.d., n=7).

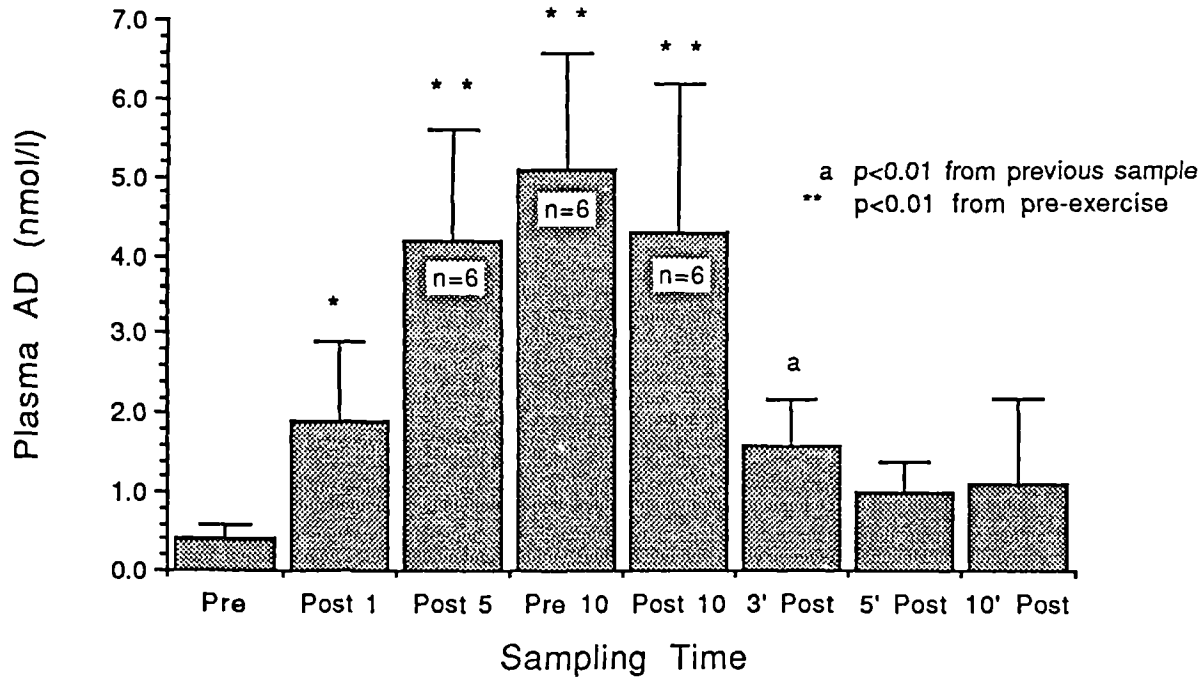


Figure 4.9. Plasma adrenaline concentrations (nmol/l) during exercise and recovery (mean ± s.d., n=7).

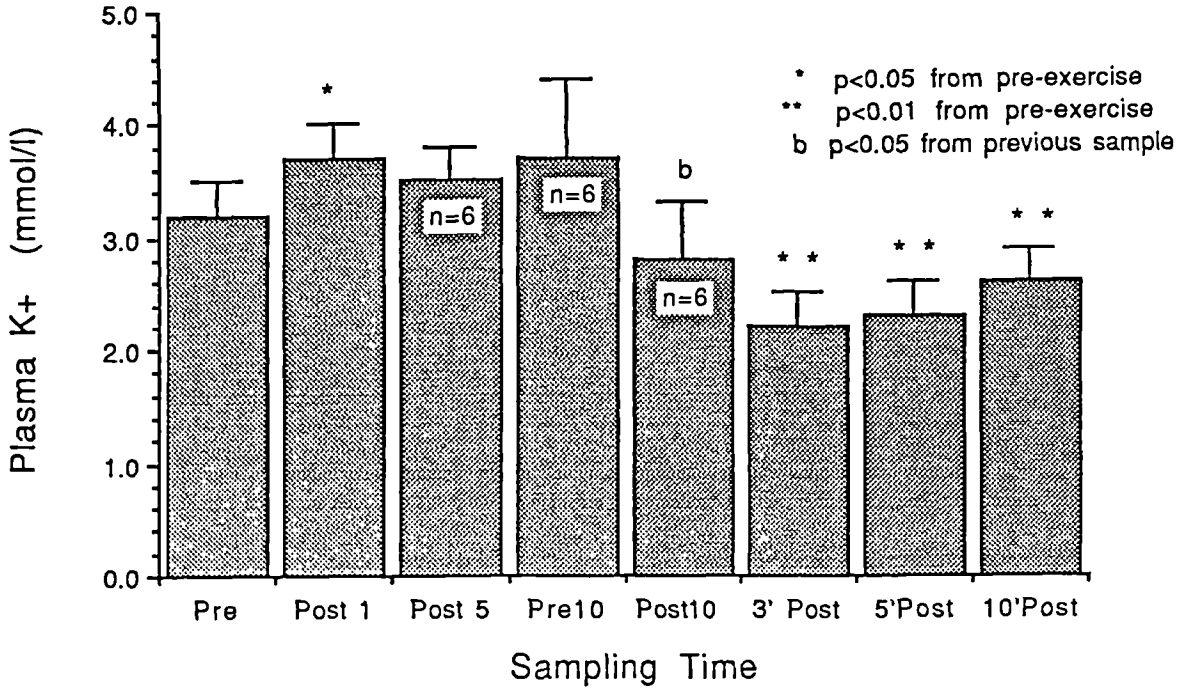


Figure 4.10. Plasma potassium concentrations (mmol/l) during exercise and recovery (mean ± s.d., n=7).

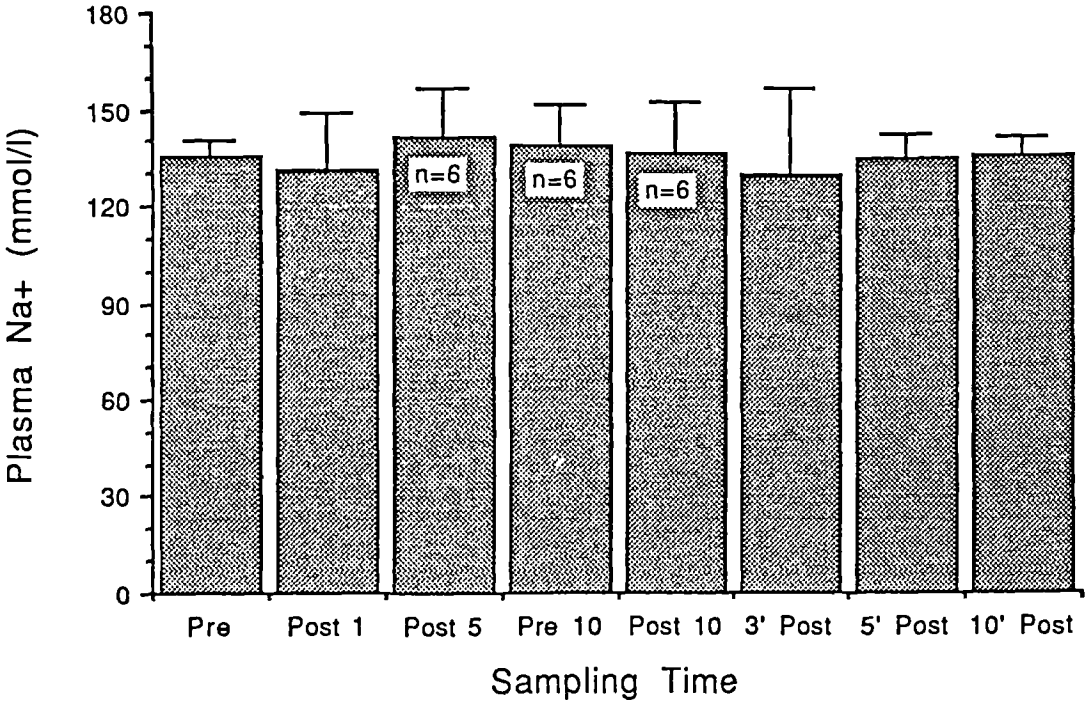


Figure 4.11. Plasma sodium concentrations (mmol/l) during exercise and recovery (mean ± s.d., n=7).

Muscle metabolites

The muscle metabolite concentrations at each sampling time are shown in Table 4.1. After the first sprint there was a 57% fall in PCr concentration ($p<0.01$) which was accompanied by a 13% fall in ATP ($p<0.01$). No accompanying changes were observed in ADP and AMP concentrations. Total glycogen concentration decreased by 14% ($p<0.01$) after sprint 1, whereas there were dramatic increases ($p<0.01$) in G-6-P (1500%), F-6-P (1100%) and lactate (653%) concentrations. There was a similar change ($p<0.01$) in F-1,6-diP (600%) but a much more modest increase ($p<0.01$) in muscle glucose content (80%) and G-1-P concentration (75%). Prior to the last sprint, the 32% decrease in ATP concentration was accompanied by a 30% fall in glycogen ($p<0.01$). Glucose-6-phosphate and F-6-P were now only 9 times greater their pre-exercise values whereas F-1,6-diP had almost fallen to resting levels. In contrast, *there was a further 3-fold increase in muscle glucose and a* further 4-fold increase in lactate compared with the values observed after the first sprint. Although the glycogen concentration fell a further 6% during the last sprint (n.s.), there was almost no change in the concentration of ATP, the glycolytic intermediates and glucose, but there was a small decrease in muscle lactate (3%, n.s.). The PCr concentration prior to sprint 10 was 51% lower than the resting value and fell to 16% during this last bout of exercise. The ADP and AMP concentrations by comparison remained unchanged. Similarly, after the initial modest increase in TP and pyruvate ($p<0.01$) observed after the first sprint, the concentration of these metabolites was maintained constant during the last sprint.

ATP production

Although some lactate efflux from muscle probably occurred during each 6s sprint, the total ATP production from anaerobic sources during the first and final sprints, respectively, was considered to be equal to the sum of $\triangle$PCr + $\triangle$ATP + 1.5 x $\triangle$lactate + 1.5 x $\triangle$Pyruvate as has been previously described (Sahlin and Henriksson, 1984). The ATP production from glycolysis was estimated from the changes in lactate and pyruvate using the formula 1.5 x $\triangle$lactate + 1.5 x $\triangle$ Pyruvate. The results of these calculations are shown in Table 4.2. Glycolysis contributed 44% of the ATP produced during the first sprint with the remainder being

TABLE 4.1 Muscle metabolites before and after the first and last 6s sprints of the multiple sprint test on the cycle ergometer (mean ± s.d., muscle glycogen in mmol.glucosyl units.kg^{-1} dm: all other values in mmol.kg^{-1} dm).

METABOLITES	PRE-1st 6s (n=8)	POST-1st 6s (n=8)	PRE-10th 6s (n=7)	POST-10th 6s (n=7)
Glycogen	316.8±74.8	273.3±79.9**	221.0±90.3[a]**	201.4±90.1**
Ac sol glyc	26.8±18.5	14.1±18.1*	7.0±15.2**	5.3±12.6**
Ac insol gl	290.0±27.1	259.3±63.3**	214.0±76.2[a]**	196.1±78.2**
ATP	24.0±2.7	20.9±2.5**	16.4±2.4[b]**	16.4±3.9**
ADP	3.0±0.4	3.5±0.6	2.7±0.4[b]	3.2±0.5
AMP	0.1±0.0	0.1±0.1	0.2±0.1	0.1±0.1
PCr	76.5±7.2	32.9±2.6**	37.5±8.0**	12.2±3.7[ac]**
Cr	43.5±4.8	87.3±12.6**	81.9±13.1**	107.3±15.0[ac]**
Glucose	1.4±0.4	2.5±1.0*	7.9±1.9[a]**	8.2±1.3**
G-1-P	0.4±0.1	0.7±0.4**	0.8±0.6	0.8±0.5
G-6-P	0.6±0.2	9.6±2.0**	5.7±1.8[a]**	5.9±1.0[c]**
F-6-P	0.2±0.1	2.4±0.9**	1.8±1.2*	1.4±0.6
TP	0.2±0.1	0.3±0.1	0.3±0.2	0.3±0.1
F-1,6-DP	0.2±0.1	1.6±1.1**	0.4±0.3[a]	0.5±0.1
Lactate	3.8±1.1	28.6±5.7**	116.2±32.9[a]**	112.3±30.6[c]**
Pyruvate	0.6±0.3	2.0±0.6**	1.6±0.3**	1.8±0.6**

a = $p<0.01$ from previous sample
b = $p<0.05$ from previous sample
c = $p<0.01$ from post-1st 6s
** = $p<0.01$ from pre-exercise
* = $p<0.05$ from pre-exercise

TABLE 4.2 Estimates of ATP production from anaerobic sources during the first and final 6s sprints of the exercise test (mean ± s.d.).

	ATP PRODUCTION ($mmol.kg^{-1}dm$)		ATP PRODUCTION RATE ($mmol.kg^{-1}dm.s^{-1}$)	
	Sprint 1 (n=8)	Sprint 10 (n=7)	Sprint 1 (n=8)	Sprint 10 (n=7)
Total	89.3±13.4	31.6±14.7**	14.9±2.2	5.3± 2.5**
From Glycolysis	39.4±9.5	5.1±8.9**	6.6±1.6	0.9±1.5**
From PCr	44.3±4.7	25.3±9.7**	7.4±0.8	4.2±1.6**

**p<0.01 from sprint 1

derived predominantly from PCr degradation. In contrast, during the last sprint anaerobic ATP production was almost entirely supplied from PCr (80%). Despite an apparent average 20% contribution from glycolysis to ATP production in the last sprint (Figure 4.12), it should be noted that in four subjects this was estimated to be zero, (range 0-23.1mmol.ATP.kg^{-1}dm, n=7).

Glycogenolytic and glycolytic rates

The anaerobic glycogenolytic and glycolytic rates were estimated using the formulae described by Hultman and Sjöholm (1983a), as follows: Glycogenolysis = ΔG-6-P + (0.33 x ΔG-6-P) + 0.5 x (Δ lactate + 0.1 x Δ lactate) and Glycolysis = 0.5 x (Δ lactate + 0.1 x Δ lactate). The results of these calculations are shown in Table 4.3. In the first sprint anaerobic glycogenolysis had exceeded glycolysis by 91% with the rate of total glycogen breakdown surpassing the rate of anaerobic glycogenolysis by almost 64%. After ten sprints, there was an 11-fold decrease in glycogenolysis and an 8-fold decrease in glycolysis although the rate of total glycogen breakdown had only fallen by 47%.

Interestingly, those individuals who had the greatest fall in MPO over the ten 6s sprints were found to have the greatest glycolytic rate during the first sprint ($r = 0.893$, $p<0.01$, $n=7$). Moreover, despite a high negative correlation between the rates of anaerobic glycogen degradation between the first and the last sprints ($r=-0.842$, $p<0.05$, $n=7$) those who achieved the greatest amount of work in sprint 1 also performed more work in sprint 10 ($r=0.851$, $p<0.05$, $n=7$).

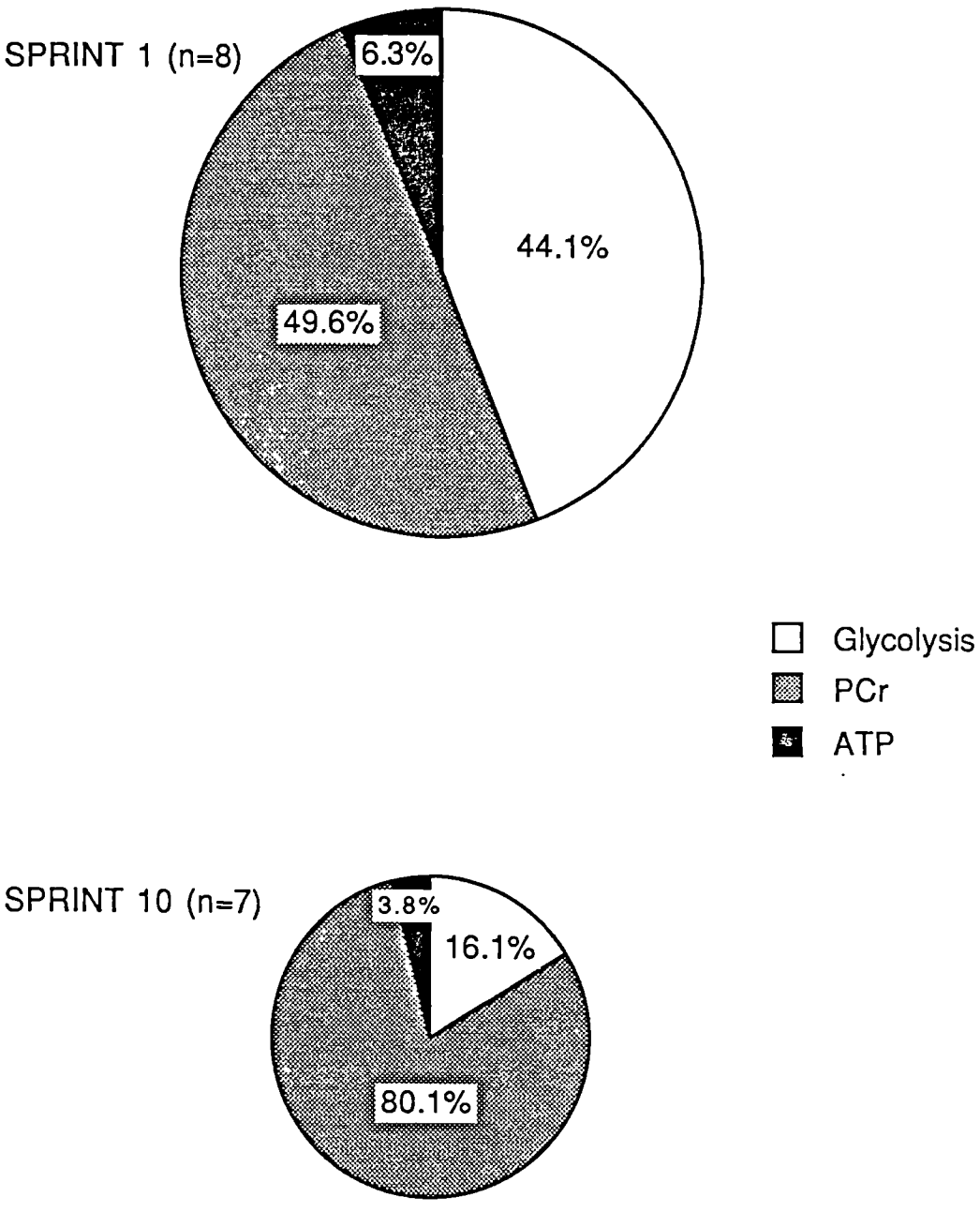


Figure 4.12. Total anaerobic ATP production.

TABLE 4.3 Estimated anaerobic glycogenolytic, glycolytic rates and rate of glycogen degradation during the first and last 6s sprints of the exercise test (mean ±s.d.).

	SPRINT 1 (n=8)	SPRINT 10 (n=7)
Glycogenolytic rate (mmol.glucosyl units $kg^{-1}dm.s^{-1}$)	4.4±0.9	0.4±0.5**
Glycolytic rate (mmol.$kg^{-1}dm.s^{-1}$)	2.3±0.6	0.3±0.5**
Rate of glycogen degradation (mmol.glucosyl units $kg^{-1}dm.s^{-1}$)	7.2±4.1	3.4±3.0**

**p<0.01 from sprint 1

4.4 DISCUSSION

Despite the brief duration of each exercise bout, there were significant decrements in the power output over the ten 6s sprints. These decrements in performance differ markedly from the findings of earlier studies that employed high-intensity, but not maximal, intermittent exercise. Margaria and co-workers (1969) showed that repeated bouts of running lasting 10s on a motorised treadmill (at 18Kmh^{-1} and 15% incline) with 30s intervals or rest, could be repeated without fatigue. Similarly, Saltin and Essén (1971) demonstrated that it was possible to perform repeated bouts of heavy cycling exercise (400W) lasting 10s, with 20s recovery intervals, for up to 30min without any obvious external signs of fatigue. However, in the above study the absolute exercise intensity was less than 50% of the average PPO generated by the subjects over the ten maximal 6s sprints, in the present study. Moreover, a load of 400W is only 60% greater than that which elicited the subjects' VO_2 max in the present study, whereas the PPO generated during the first sprint was approximately 5-fold greater.

The PPO and MPO recorded in the first sprint reached values higher to those reported previously for active male subjects performing a single bout of maximal exercise lasting 6s on a cycle ergometer. However, power output values in previous studies (Boobis et al, 1982; Wootton and Williams, 1983) have not been corrected for the initial acceleration of the flywheel (Lakomy, 1988). On isokinetic cycle ergometers higher values for PPO have been observed (McCartney et al, 1986) but these values were obtained from averaging periods of one pedal revolution, corresponding to a 0.6s interval, allowing therefore, a greater contribution from the instantaneous peak power value. The power outputs in the present study, during each exercise bout, were also higher than those values observed for active male subjects during repeated 6s bouts of sprint running with 30s recovery intervals on a non-motorised treadmill (Holmyard et al, 1988; Brooks et al, 1990). These differences in performance possibly reflect the lack of load optimisation associated with treadmill sprinting. Power output requires an optimal resistance to attain a maximal value. In treadmill sprinting this resistance is determined by the inertia of the belt and the bearings only (Lakomy,

1987). In addition only the propulsive component of the total external power output generated by the subjects is being measured during this type of treadmill sprinting (Lakomy, 1987). It is of interest, that the decline in power output observed in the present study with successive sprints was greater than the corresponding values previously reported during repeated sprints on the non-motorised treadmill. In the present study, PPO had fallen by 33.4% and MPO by 26.6% in the final sprint compared with 8.3-13.3% and 13.0-21.4%, respectively, after ten 6s bouts of sprint running (Holmyard et al, 1988; Brooks et al, 1990). The more pronounced decrements in performance observed in the present study could be possibly attributed to the greater leg speeds during cycling compared with running (Cheetham, 1987; Lakomy, 1988). Alternatively, it may be that as a result of a larger muscle mass being utilised during sprint running compared with sprint cycling, power output during sprint cycling is affected to a greater degree by local fatigue of a particular muscle group (Holmyard et al, 1988). It is also possible, that during sprint running subjects improve their running stride efficiency with increasing fatigue by shortening their leg stride (Lakomy, 1988).

The high power outputs generated by the subjects over the first sprint would require very high rates of energy production which must be provided primarily by anaerobic processes in the muscle cell. Indeed, within the first 6s bout of maximal exercise PCr concentration had fallen by 57% and total glycogen concentration had decreased by 14%. In addition, the concentrations of the glycolytic intermediates had increased several fold above their resting values. Muscle lactate concentration increased to 28.6mmol.kg^{-1} dm, confirming glycolytic activity during this brief period of maximal exercise.

The changes in the concentration of muscle metabolites were used to estimate ATP production rate. The initial ATP production was 14.9 $\pm$ 2.2mmol.kg^{-1} dm.s^{-1} which is close to the theoretical maximum rate of 17mmol.kg^{-1} dm.s^{-1} calculated by McGilvery (1975). Degradation of PCr accounted for half of the ATP resynthesised (49.6%) from anaerobic sources, the remainder was derived mainly from glycolysis (44.1%). Approximately 13% of the muscle ATP store was also directly used to support muscular contraction, although the relative energy contribution

from this store was very small (6.3%). These findings, suggest that the MPO generated over the first sprint must have been fueled by energy which was derived mainly from PCr and anaerobic glycogenolysis. These results also support the observations of other authors (Boobis et al, 1982; Jacobs et al, 1983b) that glycogenolytic processes leading to lactate formation are initiated within the first few seconds of maximal dynamic exercise. This is in contrast to previous suggestions that the muscle stores of ATP and PCr may be sufficient to meet the energy requirements of brief (10-15s) high-intensity exercise (Margaria et al, 1964; Margaria et al, 1969).

The marked fall in the intramuscular concentration of PCr after the first sprint exceeded, in absolute terms, those changes reported previously for brief (6-10s) maximal cycling (Boobis et al, 1982; Jacobs et al, 1983b) or sprint running (Hirvonen et al, 1987). However, these changes were not as great to those (80% or more) observed after exhaustive dynamic exercise lasting between 30s-3min (Hultman et al, 1967; Karlsson, 1971; McCartney et al, 1986; Nevill et al; 1989). It appears therefore, that 6s of maximal exercise may not be sufficient to permit total PCr depletion as previously suggested from theoretical calculations of some authors (Margaria et al, 1964; Newsholme, 1980). Despite this, the higher rates of PCr degradation (7.4 ± 0.8mmol.kg^{-1} dm.s^{-1}) that were observed during the first sprint in the present study compared favourably with those reported during maximal exercise lasting 30s or more. Collectively, these results indicate the importance of PCr as an energy source within the first few seconds of maximal energy demand.

The extent to which glycogen was used anaerobically in the first 6s bout of exercise, as calculated from the accumulation of hexose monophosphates and lactate + pyruvate, represents one of the highest rates of glycogenolysis (4.4 ± 0.9mmol.glucosyl units.kg^{-1} dm.s^{-1}) recorded for human quadriceps femoris muscle. This high value possibly reflects the great involvement of the vastus lateralis/quadriceps muscle group during maximal cycling. This value is similar to that observed in a previous study from this laboratory (Boobis et al, 1982). It is, however, almost twice as high as that estimated for sustained isometric

contractions at near maximal force lasting only a few seconds (Bergström et al, 1971). Because this initial rate of glycogenolysis exceeded the glycolytic rate by 1.9-fold, there were relatively larger changes in muscle G-6-P and F-6-P compared with the increases in F-1,6-diP and triose phosphates. These results are consistent with those of previous studies in which the ratio between rate of glycogenolysis and rate of glycolysis during the first 5s of sustained contraction was 2.5 (Hultman and Sjoholm, 1983a). The proportionally larger accumulation of hexose monophosphates therefore, could be explained by a greater activity of glycogen phosphorylase relative to that of PFK.

The high glycogenolytic rate calculated from changes in metabolites during the first sprint would require a total phosphorylase activity of 264mmol.glucosyl units.kg^{-1} dm.min^{-1}. This value, exceeds by 1.6 times the estimated total maximal activity of this enzyme but 5.9 times that of a determined in vitro (Chasiotis et al, 1982). This greater phosphorylase activity calculated, to that predicted in vitro, could be attributed, partly, to a higher rate and greater transformation of inactive phosphorylase b to active phosphorylase a. This could be the result of the rapid 5-fold rise in plasma adrenaline concentration that was observed after the first sprint. Indeed, adrenaline is known to increase the activity of adenylate cyclase (Newsholme and Start, 1973) which increases the concentration of cyclic AMP in muscle as has been shown to occur during intense dynamic exercise (Chasiotis et al, 1983a).

The presence of a high concentration of Ca^{2+} in the sarcoplasm, as a result of the maximal exercise, could also augment the conversion of phosphorylase b to a by increasing the activity of phosphorylase b kinase (Newsholme and Start, 1973; Chasiotis, 1988). However, it has been suggested that the availability of Pi may be a more important regulatory factor to phosphorylase activity during muscular contraction, as phosphorylase activity could remain at a low level even if transformation of b to a occurred (Chasiotis et al, 1982; Chasiotis et al, 1983b). In the present study, the first sprint must have resulted in a rapid increase in intracellular Pi concentration as indicated by the high rate of PCr degradation. Although, the intracellular phosphate concentration during the initial 6s bout of exercise was not measured,

its concentration as determined from the changes in ATP, ADP, PCr and hexose monophosphates (Bergström and Hultman, 1988) is estimated to be 40mmol.kg^{-1} dm or 15.5mmol.l^{-1} intracellular water, assuming a Pi concentration of 2.9mmol.l^{-1} at rest (Chasiotis, 1983). This value is similar to that observed after 10s of intermittent electrical stimulation at 20Hz (Chasiotis et al, 1987), yet the calculated glycogenolytic rate in that study was 2.8 times less compared with that observed in the present study. However, the sensitivity of phosphorylase a for Pi is increased with elevated AMP concentrations (Chasiotis et al, 1982) which could be a possible explanation for the high glycogenolytic rates seen in the first sprint. Following completion of the first sprint AMP concentration did not change from resting values, possibly because the rapid transient increases in AMP are difficult to determine in muscle samples obtained by muscle biopsies (Sahlin, 1986). It is also possible that AMP deaminase was activated within the first few seconds of maximal exercise owing to the high ATP turnover rate coupled with a considerably reduced PCr level that was observed. This would promote continued formation of ATP from ADP and thus keep the ATP to ADP ratio high. However, the AMP concentration early in maximal contraction is probably sufficient to decrease the Km for Pi but not to activate the b form of the enzyme (Chasiotis, 1983).

The possibility that the high glycogenolytic rate calculated for the first sprint was overestimated due to the fact that the pre-exercise muscle sample was taken prior to the warm-up seems unlikely. Within the first 6s bout of exercise muscle lactate was 28mmol.kg^{-1} dm, a value which is similar to that observed after a maximal cycling test of the same duration, in which however, the pre-exercise muscle sample was taken after the warm-up (Boobis et al, 1982). Because the warm-up in the present study was of short duration and submaximal in nature, it is evident from the data of Boobis and co-workers that its contribution to the decrease in muscle glycogen stores was quite small (approximately 3mmol.glucosyl units.kg^{-1} dm). If one considers the measured fall in muscle glycogen during the first 6s sprint it can be seen that 43.5mmol.glucosyl units.kg^{-1} dm were broken down, corresponding to an estimated glycogen degradation rate of 7.2mmol.kg^{-1} dry wt.s^{-1}. This rate is 1.6 times higher than that calculated from the changes in hexose

monophosphates and the accumulation of lactate and pyruvate. However, even if one accounts for all the changes in the muscle glycolytic intermediates, lactate, pyruvate, free muscle glucose and blood lactate and glucose concentrations measured in the present study, still 13mmol.glucosyl units.kg^{-1} dm remain unaccounted for. Although the changes in the concentrations of muscle glycerol-3-phosphate and alanine were not determined, it seems unlikely that these metabolites could account for 30% of the total glycogen mobilised during the first 6s sprint. There is of course the possibility that some glycogen was used aerobically. However, assuming an ATP energy provision at 100KJ/mole (McGilvery, 1975), a 20% mechanical efficiency (Kavanagh and Jacobs, 1988), a water content of 77% and an exercising muscle mass of 14.4Kg for the subjects used in this study [muscle mass of the lower extremeties calculated from radiogrametric estimates of lean leg size in litres (Wootton, 1984) and an average leg density of 1.0$Kg.l^{-1}$ (Dempster, 1955)] and applying this figure to the total work done in the 6s sprint, it can be estimated that a total ATP production rate of 13.1$mmol.kg^{-1}$ $dm.s^{-1}$ was required to sustain the work done. This value is remarkably close to that calculated in the present study from anaerobic sources. Another approach, supporting the view that most of the glycogen was metabolised anaerobically, is to examine the relationship between glycogen utilisation and lactate produced. Interestingly, those individuals who exhibited the highest rates of glycogen degradation in the first sprint accumulated more lactate in muscle ($r=0.793$, $p<0.05$, $n=8$) and also had the greatest ATP production rates from anaerobic sources ($r=0.837$, $p<0.01$, $n=8$).

Unlike earlier studies which examined PCr concentration following dynamic exercise (Bergström et al, 1971; McCartney et al, 1986) and electrically stimulated muscle contractions (Hultman and Sjoholm, 1983a; Chasiotis et al, 1987), no total depletion (i.e. less than 10% of resting values) was found in the present study following completion of the final sprint. The PCr concentration measured 10s prior to the tenth sprint was still only 49% of the resting value and subsequently fell to 16% during the last sprint. However, if one assumes that PCr was totally depleted after completion of the ninth sprint and that a maximal 5s period was elapsed from muscle sampling to muscle freezing, then it

can be calculated from the observed PCr values measured 10s prior to the last sprint, that PCr concentration was restored at a rate of 1.5mmol.kg^{-1} dm.s^{-1}, which is almost in close agreement with the value of 2mmol.kg^{-1} dm.s^{-1} reported by Harris and co-workers (1976) following exercise with intact circulation. Based on these calculations it appears that the muscle PCr stores were reduced almost to the same extent during the first and the final sprints. If this was the case, then it suggests that a 30s recovery interval may be sufficient to allow significant PCr resynthesis and therefore considerable contribution from PCr to ATP production during this type of intermittent exercise, even after ten maximal 6s sprints. This is in contrast to previous suggestions that PCr degradation may be of minor importance in maintaining ATP production rates high during the latter stages of this particular intermittent exercise test (Wootton and Williams, 1983; Holmyard et al, 1988).

Of course, it is recognised that this fast rate of PCr resynthesis calculated here, could be an overestimation owing to the effect of a low intramuscular pH upon the creatine kinase equilibrium (Sahlin et al, 1975). It has been suggested however, that the fast component of PCr resynthesis may be limited by the availability of oxygen rather than a low intramuscular pH (Harris et al, 1976; Sahlin et al, 1979). Nevertheless, data from studies obtained following sustained isometric contractions indicate rates of PCr resynthesis to be of an order of 0.4-0.5mmol.kg^{-1} dm.s^{-1} for the first 60s of recovery with full circulation (Ren et al, 1988; Hultman et al, 1981).

Previous work from this laboratory has proposed that intermittent maximal exercise of brief duration (6s) with short intervals of rest (30s) would lead to an increasing demand on non-oxidative glycolysis to maintain the rate of energy production as a result of incomplete resynthesis of the PCr stores (Wootton and Williams, 1983). This was not seen in the present study which employed a similar work to rest ratio. In the final 6s of exercise MPO was still 73% of that in the initial sprint but the estimated ATP production rate from anaerobic sources was markedly reduced to 35.6% (5.3 $\pm$ 2.5mmol.kg^{-1} dm.s^{-1}). Glycolysis contributed only 16.1%, as the glycolytic rate had fallen 7.6

times whereas, the contribution from PCr to the anaerobic ATP production was now 80.1%. In addition there was a 10-fold decrease in the glycogenolytic rate although it always exceeded glycolysis. These findings are in contrast to those reported previously that the glycolytic rate measured as lactate formation was not reduced even after 50s of electrically induced isometric contractions and that the energy required to sustain the last 30s of contraction was derived from glycolysis (Hultman and Sjoholm, 1983a). Therefore, the decline in power output in the present study, was accompanied by evidence of decreasing contribution to ATP production from glycogen degradation to lactate formation, as indicated by the changes in muscle hexose monophosphates and lactate concentrations before and after the last exercise bout.

The increase in muscle lactate concentration found at the end of the exercise test has been shown to be associated with large falls in muscle pH (Hultman and Sahlin, 1980). It seems likely that acidosis resulting from maximal anaerobic degradation of glycogen, particularly at the early stages of the test, may be responsible for the observed reduction in the glycogenolytic and glycolytic rates. Blood pH decreased to 7.10 after completion of the test. In other studies blood pH has been shown to decrease to 7.05 after ten 6s sprints of sprint running (Brooks et al, 1990) and to 7.10 after a 400m sprint on the track (47-56s) (Costill et al, 1983). These blood pH values have been related to decreases in muscle pH of about 6.6 units. This muscle pH value is widely reported as occurring at exhaustion (Hermansen and Osnes, 1972; Sahlin et al, 1976; Sahlin 1978; Bell and Wegner, 1988). Using the equation described by Sahlin (1978) for calculating muscle pH from muscle lactate and pyruvate concentration and applying it to the values obtained in the present study, it is estimated that the muscle pH prior to the last sprint was 6.59.

The consequences of such a low muscle pH are several. The activity of the two key enzymes (phosphorylase and PFK), known to play an important role in the regulation of the anaerobic degradation of glycogen to lactate formation, is pH sensitive. In human muscle a retransformation of phosphorylase a to b has been observed during dynamic and isometric

exercise (Chasiotis et al, 1982). This decrease in phosphorylase a has been found to be below resting levels after dynamic exercise to exhaustion. It is possible therefore, that the hormonal activation of the phosphorylase system, which is mediated by cyclic-AMP, was influenced by a low pH which would favour a reduction in phosphorylase b kinase activity, thus lowering the percentage of phosphorylase in the a form. Indeed, it has been shown in vitro that the activity of adenylate cyclase decreases when pH decreases below 7.0 (Danforth, 1965). In the present study the reduction in the rate of glycogen degradation is observed despite the high adrenaline concentration found in the last sprint. Because the variation in adenylate cyclase activity in the physiological pH region is small (Newsholme and Start, 1973), it is probably not important in the regulation of the glycogenolytic rate at a low intramuscular pH.

The reduction in the glycogenolytic rate could possibly be better explained as the effect of a decrease in the concentration of the unprotonated form of Pi at a low pH which is the substrate for glycogen phosphorylase (Chasiotis, 1983). In addition to the formation of organic phosphates which lowers the availability of Pi for glycogenolysis a decrease in muscle pH to 6.60 would almost result in a transfer of 50% of Pi in the diprotonated form ($H_2PO_4^-$) (Chasiotis, 1983). Another possible reason for the reduced rate of glycogenolysis could be the decrease in the concentration of Ca^{2+} release from the sarcoplasmic reticulum. At a low pH an increased binding of Ca^{2+} to the sarcoplasmic reticulum has previously been shown (Nakamura and Swartz, 1972). A depressed Ca^{2+} release in intact fibres in the same situation has also been suggested (Fabiato and Fabiato, 1978). Moreover, in human wrist flexor muscles increased concentration of $H_2PO_4^-$ has been closely related to the decline in force development (Wilson et al, 1988). If this is the case (i.e. a high concentration of $H_2PO_4^-$ and decreased Ca^{2+} release), then inhibition of the contractile mechanism and thus of ATP hydrolysis would be the primary result at a low intramuscular pH. Consequently, the decreased rate of ATP resynthesis observed in the last sprint as a result of a low rate of glycogen degradation to lactate would be secondary to inhibited ATP hydrolysis (Hultman et al, 1987). The retransformation of phosphorylase a to b has also been found to

parallel a decrease in contraction force (Chasiotis, 1983). This would further support the aforementioned hypothesis and could explain the fatigue induced decrements in performance seen in the present study, particularly if decreased activation of glycogenolysis occurred primarily in type-II fibres (approximately 50% of vastus lateralis) in which the maximal activity of phosphorylase is 2-3 times greater than in type-I fibres (Harris, Essén and Hultman, 1976).

The marked fall in the concentration of hexose monophosphates prior to the last sprint provides further evidence that anaerobic glycogen degradation was inhibited at the flux generating step controlled by phosphorylase. Alternatively, the accumulation of hexose monophosphates during successive sprints was reduced because they could have been used as a possible substrate for the resynthesis of PCr via oxidative metabolism during the recovery intervals. Resynthesis of PCr during recovery, after consumption of any free Pi present, may be linked to the decay of these phosphates.

Earlier human studies have reported substantial glycolytic activity despite large increases in muscle acidity (Hultman and Sjoholm, 1983a; Spriet et al, 1987a). These results suggest that the net effect of all inhibitors and activators which regulate the activity of PFK (Dobson et al, 1986), broadened the pH profile of the enzyme into a physiological range which in vitro studies have found to be inhibitory (Danforth, 1965; Ui, 1966). In the present study the glycolytic rate, estimated from lactate accumulation, in the last 6s of exercise was decreased, indicating a reduction in the activity of PFK enzyme. Moreover, owing to a considerable reduction in the rate of glycogenolysis, the unchanged concentrations of hexose monophosphates relative to F-1,6-diP and triose phosphates before and after the last sprint, further point to PFK inhibition as has been previously suggested (Sahlin, 1978). These observations suggest that in the last 6s of exercise the glycogenolytic flux was almost equal to the glycolytic flux. This is in contrast with the first sprint, where a relatively larger increase of hexose monophosphates to F-1,6-diP occurred, suggesting a massive overstimulation of glycogen phosphorylase relative to PFK. This difference in the activities of the two enzymes was probably caused by

the differences in the intracellular environment and cannot be explained from the plasma adrenaline concentrations. Following completion of sprint 9, plasma adrenaline concentration reached its peak value (5.1 $\pm$ 1.5nmol.l^{-1}). This value was almost 3-fold higher to that found after sprint 1.

In spite of a dramatic reduction in energy yield from anaerobic glycogenolysis in the last sprint of exercise, the MPO achieved was 73% of that in the initial sprint. Several possibilities exist to explain this difference. Firstly, a greater reliance on aerobic metabolism of glycogen is possible. A detectable fall in glycogen content was observed in the last bout of exercise which cannot be accounted for by the accumulation of glycolytic intermediates, glucose and lactate. It is difficult to account for the amount of lactate that escaped from the muscle into the circulation during that sprint. The increase in blood lactate immediately after exercise may reflect glycogenolytic activity of all preceding exercise bouts. As no differences in blood lactate however, were seen before and after the last exercise bout, it is possible that little lactate left the muscle during this period of exercise. Moreover, the post-exercise hyperglycaemia in blood observed in the present study is thought to have derived from liver glycogenolysis due to the high circulating levels of catecholamines. These high concentrations in plasma adrenaline and nor-adrenaline compare favourably with those found during ten 6s sprints on a non-motorised treadmill (Brooks et al, 1990). An increase in glucagon after maximal intermittent exercise has also been reported (Naveri et al, 1985).

The possibility therefore that some glycogen was used aerobically in the last sprint cannot be excluded as elevated levels in H^+ concentration have been suggested to increase the activity of pyruvate dehydrogenase and so enhance aerobic metabolism (Newsholme and Leech, 1983). It has been shown that during 60s of uphill treadmill running to exhaustion, the contribution from aerobic metabolism to total energy expenditure can be as high as 28-37% (Thomson and Garvie, 1981). Moreover, the intermittent studies of Essén (1978) and Essén and Kaisjer (1978) have shown that intramuscular triacylglycerol stores may be a potential fuel

source during this type of exercise. The 500% increase in plasma glycerol observed in a recent study in the last bout of maximal intermittent exercise was attributed to lipolysis in muscle (McCartney et al, 1986). If a relatively high rate of fat oxidation did occur in the present study, then the resulting elevated levels of citrate via the citric acid cycle would be expected to contribute to the reduction of PFK activity, as has been previously shown (Jansson, 1980).

A significant shift to aerobic metabolism at the latter stages of the exercise test, due to a reduced anaerobic energy yield, seems to be the most plausible explanation for the decrease in power generated during the last sprint. Recruitment of additional muscle groups that usually play a secondary role in power generation seems unlikely owing to the maximal nature of the exercise test. An increase in efficiency of muscular contractions is also unlikely as efficiency during dynamic exercise appears to decrease when lactate accumulates (Luthanen, Rahkila, Rusko and Vitasalo, 1987), in contrast to continuous stimulation where the energy cost per unit force is decreased (Spriet et al, 1988).

Muscle ATP concentration was reduced by 32% from rest after performing ten 6s sprints. Several studies have reported similar reductions in ATP concentration following intense exercise despite the marked diversity of different exercise models, absolute exercise intensities and durations employed. However, all the studies have failed to show any major depletion of this metabolite with exercise (Boobis et al, 1982; McCartney et al, 1986; Cheetham et al, 1986; Spriet et al, 1987a). It would appear that a severe reduction in muscle ATP concentration may be controlled so that it does not fall below a critical level. Depletion of muscle ATP concentration may result in irreversible damage of the contractile mechanism as has been shown to occur in rat muscle poisoned with iodoacetate (Sahlin et al, 1981). It seems unlikely therefore, that the reduced muscle content of ATP observed at the end of exercise was the limiting factor per se for the fatigue induced decrements in power output in the present study. However, the possibility that a large decrease in ATP content could have been restricted to type-II fibres, as previously suggested (Meyer et al, 1980; Meyer and Terjung,

1979) cannot be excluded as this would result in fewer cross-bridge attachments in those fibres. It is of interest to note that strong negative correlations were found between the concentration of ATP and lactate after the first sprint ($r=-0.845$, $p<0.01$, $n=8$), prior to the last sprint ($r=-0.992$, $p<0.01$, $n=7$) and immediately post-exercise ($r=0.861$, $p<0.05$, $n=7$). Therefore, those who experienced the greatest acidosis in muscle exhibited the lowest concentrations in ATP. These results may suggest that a decrease in muscle pH, as reflected by the accumulation of muscle lactate at the end of exercise, may be of importance for the activation of AMP deaminase in addition to a high requirement for energy production. In vivo, AMP deaminase is considered to be activated by increased levels of ADP, AMP and H^+. In addition, studies have shown that during exercise muscle IMP accumulation does not occur until muscle lactate reaches 50-75mmol.kg^{-1}dm (Dudley and Terjung, 1985; Harris and Hultman, 1985).

Moreover, in the present study fatigue was not associated with depletion of muscle glycogen. Although, glycogen degradation occurred at a rapid rate, it was unable to proceed to the point where glycogen stores were limited. This result is in contrast with previous suggestions that during intermittent exercise glycogen depletion may be a contributory factor to fatigue (MacDougall et al, 1977; Thomson, Green and Houston, 1979). After ten 6s sprints muscle glycogen was reduced by only 36% which supports the findings of other authors that during exercise of high-intensity and brief duration subjects became exhausted before glycogen depletion occurred (Hermansen and Vaage, 1977). It is open to speculation however, whether the decline in performance seen in the present study is partly attributed to an early depletion of glycogen in highly recruited type-II fibres. As with ATP, glycogen depletion may be localised and may not be reflected in altered whole muscle metabolite concentration (Edgerton, Saltin, Essén and Simpson, 1975; Gollnick, Karlsson, Piehl and Saltin, 1974).

Potassium loss from muscle during intense dynamic exercise is well documented (Sjogaard, 1983; Medbo and Sejersted, 1985; McKenna, Heigenhauser, McKelvie, Sutton, MacDougall and Jones, 1989). A potassium efflux is thought to be associated with a low membrane

resistance in metabolically fatigued muscle (Grabowskie, Lobsiger and Luttgau, 1972). These speculations have been supported in recent studies that have shown that the K^+ efflux may well be due to mechanisms other than those associated with the action potential. It has been reported that ATP-dependent K^+-channels have been identified in skeletal muscle (Spruce, Standen and Stanfield, 1985). These observations suggest that membrane K^+ conductance may be increased by metabolic changes within the muscle fibre, for instance ATP depletion, and thus impair action potential propagation and consequently the development of force. The increase in plasma K^+ concentration found in the present study does not appear to be of sufficient magnitude (Sjoggard, 1989) to effect the excitability of the sarcolemma. It is possible however, that K^+ accumulation could be greater in the T-tubules where diffusion is restricted due to the high surface to volume ratio (Bigland-Ritchie et al, 1978; Almers, 1980), in which case, propagation of action potentials to the interior of the cell might have been seriously affected. The rapid recovery seen in plasma K^+ concentration post-exercise, may reflect an increment of the Na^+/K^+ pump and other re-uptake mechanisms (Sejersted and Hallen, 1987).

In summary, the results of the present study indicate that the average power output generated over the first sprint was supported by energy which was derived mainly from PCr and anaerobic glycogenolysis. In the final sprint, however no lactate accumulation was apparent, yet the average power output was still 73% of that in the initial sprint. In face of an apparent marked reduction in the rate of anaerobic glycogenolysis, at the end of exercise, it is suggested that the energy was derived from PCr degradation and oxidative metabolism.

5. INFLUENCE OF ALKALOSIS ON PERFORMANCE AND MUSCLE METABOLISM DURING INTERMITTENT EXERCISE

5.1 INTRODUCTION

There is increasing evidence that the accumulation of hydrogen ions within the muscle and the associated intracellular acidosis may partly account for the observed reduction in performance during a short period of high-intensity exercise. Previous studies have suggested that increasing the buffering capacity potential of the blood, by ingesting bicarbonate solutions prior to exercise, may enhance the efflux of hydrogen ion from the muscle to the systemic circulation. Since the bicarbonate ion is the major buffer of the blood, it may be possible to delay the fall in intramuscular pH, by increasing the rate of translocation of H^+ from muscle to blood. Thus, the onset of fatigue may be delayed by increasing the alkali reserve prior to high-intensity exercise. In previous studies on human subjects, significant improvements in running performances have been recorded (Wilkes et al, 1983; Goldfinch et al, 1988). However, these studies simply implied from circumstantial evidence that the improvements in performance were the result of the acid-base changes in muscle and blood following the ingestion of bicarbonate solutions.

The aim of the present study was to establish whether or not the changes in performance during maximal intermittent exercise were accompanied by favourable changes in muscle pH and muscle metabolism after ingesting solution of sodium bicarbonate. In the present study, the amount of work done per sprint, rather than the duration of the sprint is standardised, irrespective of experimental condition. In doing so, it is possible to compare the metabolic changes that occur after a given number of sprints over which however, the total amount of work accomplished is the same, irrespective of experimental trial.

5.2 METHOD

Subjects

Eight healthy male physical education students volunteered to take part in this study which had the approval of the University Ethical Committee. The mean ($\pm$ s.d.) age, height and weight of the subjects were 24.9 $\pm$ 2.7 years, 175 $\pm$ 6.8cm and 68.8 $\pm$ 7.1Kg, respectively. The experimental protocol was fully explained to the subjects and a written informed consent was obtained.

Protocol

Subjects completed two identical tests separated by seven days. On each occasion, at least four hours after the last meal, the subjects ingested a solution of either sodium bicarbonate ($NaHCO_3$) or sodium chloride (NaCl) 2.25 hours prior to exercise. The doses were $0.3g.Kg^{-1}$ body weight for the alkaline treatment and 1.5g in total for the placebo (NaCl). Both the $NaHCO_3$ and the NaCl were dissolved in 500ml of water and consumed within a 10min period. The taste of both solutions was very similar and so subjects were not able to distinguish between the two solutions. The order of testing was randomly assigned and the experiment was conducted in a double-blind manner. An initial pilot study had shown that blood bicarbonate concentration, base excess and blood pH were significantly elevated 2.25h after drinking the bicarbonate solution (Appendix C).

The exercise test, referred to as the 'constant-work multiple-sprint test', consisted of repeated maximal sprints, with 30s recovery between sprints, performed on an instrumented cycle ergometer. During each exercise bout subjects were required to perform a given amount of work which was equal to the highest they attained in a series of single 6s sprints conducted over a 5 days period, the last session being at least 3 days prior to testing. As the test progresses the time taken to complete the standardised amount of work increases with each subsequent period of exercise (Lakomy, 1988). When the time required to complete the predetermined work exceeded 10s the exercise test was terminated (Figure 5.1). The initial familiarisation sessions indicated that all subjects were capable of completing at least six maximal sprints with

30s recovery in less than 10s each and still reaching maximal speeds above 90rpm, a value close to the reported optimum pedalling speed (McCartney et al, 1983a; Jones et al, 1985). In addition to the time taken to achieve the set amount of work, PPO was also monitored and recorded throughout the experiment. Heart rate was monitored continuously during the exercise-test and for the first 15min of recovery after exercise.

Maximal oxygen uptake was determined on the cycle ergometer on a separate occasion, as previously described (see Chapter 3, for methods).

Blood and muscle sampling

Venous blood samples were taken at rest prior to the ingestion of the bicarbonate solution, *15min prior to exercise*, *immediately after the* fifth and final sprints and at 3min, 5min, 10min and 15min of recovery following completion of the last sprint. Venous samples were analysed immediately for blood pH and carbon dioxide partial pressure (PCO_2) and at a later date for blood lactate, glucose and plasma catecholamine concentrations. Blood bicarbonate, base excess and standard bicarbonate concentrations were determined using a Severinghaus slide ruler. Blood oxygen partial pressure (PO_2) was also measured in the pre-test sample only to document the extent of arterialisation of venous blood and was not used in subsequent analysis. The mean ($\pm$ s.d.) PO_2 was 48 $\pm$ 8.7 Torr, with no significant differences between acid-base states. Changes in plasma volume were estimated from the immediate post-exercise haematocrit and haemoglobin values (Dill and Costill, 1974).

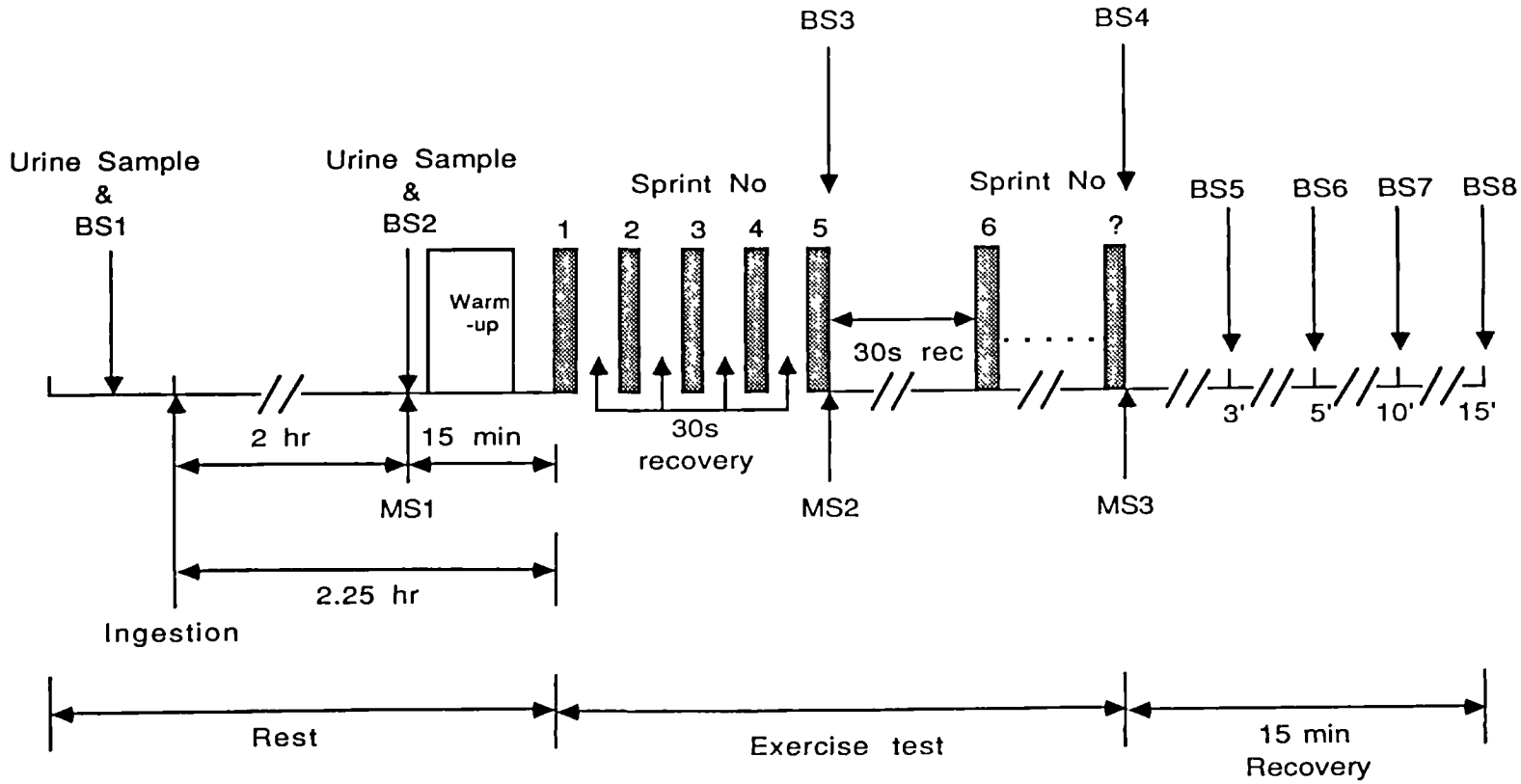
Muscle samples were taken from the vastus lateralis muscle of one leg at rest 15min prior to testing, immediately after the fifth sprint and immediately after termination of the final sprint. On each occasion muscle samples were taken from a different leg and the order of selection was randomly assigned. Muscle samples were removed from the biopsy-needle in liquid nitrogen and split into two pieces. One portion was freeze-dried for muscle metabolite analysis, whilst the remainder was stored in liquid nitrogen for subsequent determination of pH on muscle homogenates and buffering capacity by HCl-titration (see Chapter 3, for methods).

Urine sampling

Urine samples were collected prior to ingestion of $NaHCO_3$ or NaCl and within 30-15min prior to the resting muscle-biopsy sample. Samples were kept refrigerated at $4^{o}C$ and analysed for pH within 1 hour following completion of the test.

Statistical analysis

A paired T-test was used to examine the differences in performance variables between conditions and a two-way analysis of variance with repeated measures on two factors (experimental condition and sampling time) was performed on the muscle and blood metabolite data. A Pearson product moment correlation was used to examine the relationship between variables. Statistical significance was accepted at the 5% level. Results are presented as mean $\pm$ s.d.



BS = Blood Sample
MS = Muscle Sample

Figure 5.1. Schematic illustration of study's protocol.

5.3 RESULTS

Performance results

The amount of work subjects performed during each sprint was 4886 ± 518J in both experimental conditions and the time required to complete this standardised amount of work in the first exercise bout was 6.3 ± 0.5s in the placebo and 6.2 ± 0.5s in the alkaline trials (n.s.). Although, the number of sprints completed between subjects was unequal owing to the open-ended nature of the exercise test, all subjects (n=8) were capable of completing at least seven sprints within 10s, regardless of condition.

The PPO achieved and the time required to complete each of the first seven sprints (n=8) are shown in Figures 5.2 and 5.3, respectively. As the tests progressed, considerable decrements in PPO occurred between sprints ($p<0.01$) whilst, the time required to perform the standardised amount of work increased with each subsequent exercise bout ($p<0.01$), irrespective of condition. Following placebo and alkaline treatment, PPO reached its highest value in the first sprint (1157.8 ± 137.2W and 1127.9 ± 144.5W, respectively, n.s.). These values were over four times that which elicited the subjects VO_2 max (254.1 ± 26.1W). The PPO values decreased by 44% in the placebo and 42% in the alkaline condition during the final sprint (n.s. between conditions). No statistically significant difference in PPO and time required to complete the set amount of work was found at any individual sprint of the exercise test as a function of the experimental conditions. In addition, the total time taken by the subjects to complete the same amount of work in the first five sprints (i.e. average value = 4886W x 5) was not different between conditions (35.7 ± 2.4s, placebo vs 35.1 ± 2.0, alkaline; n.s.). However, following completion of sprint 5, it was evident that subjects were able to exercise for a further 55.1 ± 25s in the alkaline compared with 50.2 ± 28.7s in the placebo condition (9.8% longer in alkaline, n.s.). Consequently, subjects accomplished more work during this time which was 14.1% greater compared with that attained in the placebo condition (n.s.), (Table 5.1). It should be noted however, that these differences in performance, between the two conditions, are considerably

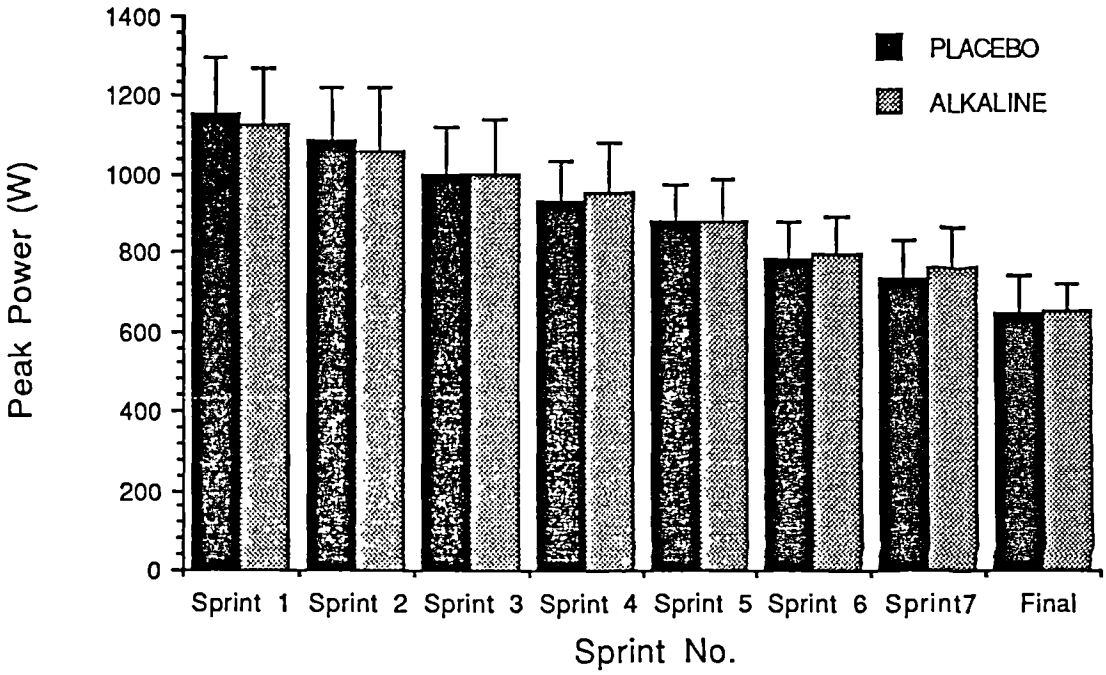


Figure 5.2. Peak power output (W) for each of the first 7 and final sprints in the placebo and alkaline conditions (mean ± s.d., n=8).

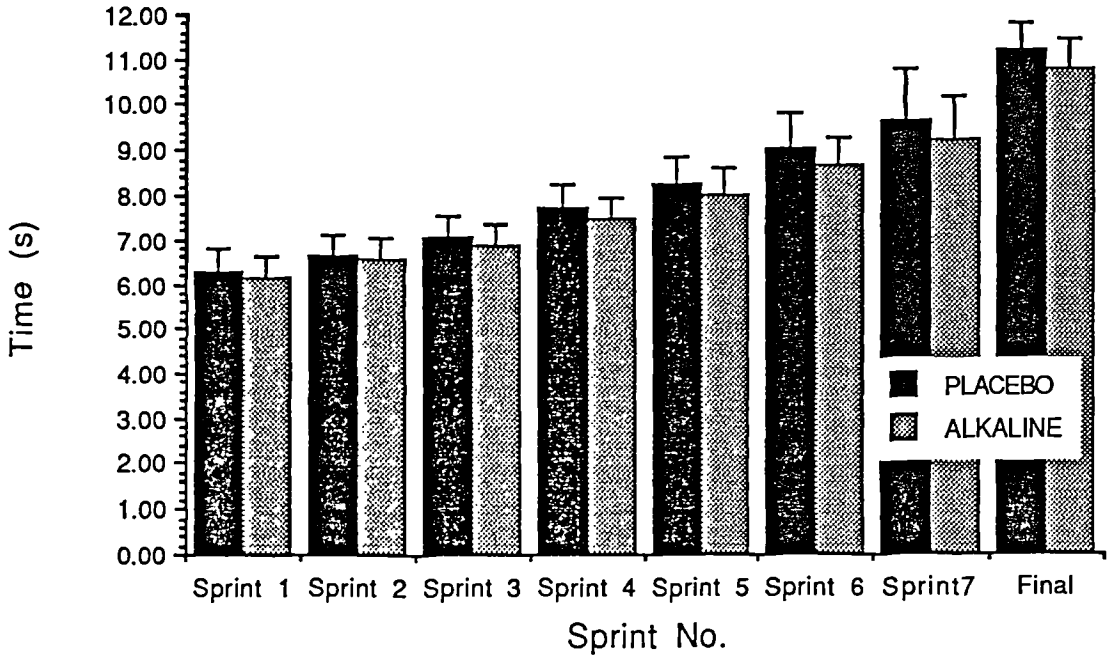


Figure 5.3. Time (s) taken for each of the first 7 and final sprints in the placebo and alkaline conditions (mean ± s.d., n=8).

TABLE 5.1 Performance indices in the placebo and alkaline conditions (mean ± s.d., n=8)

VARIABLE	EXPERIMENTAL CONDITION Placebo	Alkaline	% CHANGE IN ALKALINE	p LEVEL
Total time(s) Sprint 1-5	35.7 ±2.4	35.1 ±2.0	-1.7%	n.s.
Total time(s) Sprint 6-final	50.2 ±28.7	55.1 ±25.0	+9.8%	n.s.
Total time(s) Sprint 1-final	85.9 ±29.2	90.2 ±25.9	+5.0%	n.s.
Total work done(J) Sprint 6-final	24401.3 ±13965.3	27838.4 ±13233.2	+14.1%	n.s.
Total work done(J) Sprint 1-final	48787.4 ±13599.0	52223.3 ±13665.1	+7.0%	n.s.
Average work rate(W) Sprint 1-5	691.2 ±76.5	703.3 ±76.5	+1.8%	n.s.
Average work rate(W) Sprint 6-final	489.0 ±50.5	504.2 ±49.1	+3.1%	n.s.
Average work rate(W) Sprint 1-final	600.3 ±67.0	605.2 ±61.0	+0.8%	n.s.
ΔAverage work rate(W) Sprint 1-final	347.9 ±83.2	344.8 ±88.4	-0.9%	n.s.
ΔPPO(W) Sprint 1-final	510.0 ±133.7	476.0 ±137.9	-6.7%	n.s.

reduced if expressed as work performed (J) over unit of time (s). In doing so, the average rate (average MPO) from sprint 5 to the final sprint was 3.1% greater in the alkaline condition compared with the placebo condition (n.s.) and only 0.8% greater for the entire test (n.s.), (Table 5.1).

Blood pH, lactate and buffering capacity

Following the ingestion of the bicarbonate solution resting pre-exercise venous blood pH was elevated above placebo values (7.42 $\pm$ 0.04 vs 7.34 $\pm$ 0.03, $p<0.01$) (Figure 5.4), indicating that a condition of metabolic alkalosis had been achieved. This condition was also confirmed by the higher HCO_3^- concentration in blood (25.6 $\pm$ 1.6mmol.l^{-1} vs 22.3 $\pm$ 2.2mmol.l^{-1}, $p<0.01$) (Figure 5.5). Higher blood base excess (Figure 5.6) and standard HCO_3^- concentrations (Figure 5.7) were also observed prior to exercise following $NaHCO_3$ administration ($p<0.01$). Standard HCO_3^- concentration was calculated to enable the pure effect of increased HCO_3^- to be examined without the influence of varying degrees of respiratory compensation.

Pre-exercise blood lactate concentration was unaffected by the ingestion of $NaHCO_3$, but in all other cases during exercise and recovery, values were greater than those obtained in the placebo condition ($p<0.05$) (Figure 5.8). Blood lactate concentration was different from resting after sprint 5, in both conditions and reached their highest values at 5min of recovery which were 15.5 $\pm$ 1.3mmol.l^{-1} in the placebo trial and 17.1 $\pm$ 1.9mmol.l^{-1} in the alkaline trial ($p<0.01$). Blood lactate concentration at 10min post-exercise was found to be most highly correlated with the total work done over the entire exercise period ($r=-0.888$, $p<0.01$; placebo and $r=-0.716$, $p<0.05$; alkaline). The highest correlation between blood lactate and blood pH was found after sprint 5 in both conditions ($r=-0.827$, $p<0.05$; placebo and $r=-0.929$, $p<0.01$; alkaline).

Blood pH and base excess values were also lower in all cases than the pre-exercise value ($p<0.01$), but always higher in the alkaline condition compared with the placebo ($p<0.05$), both during and after completion of

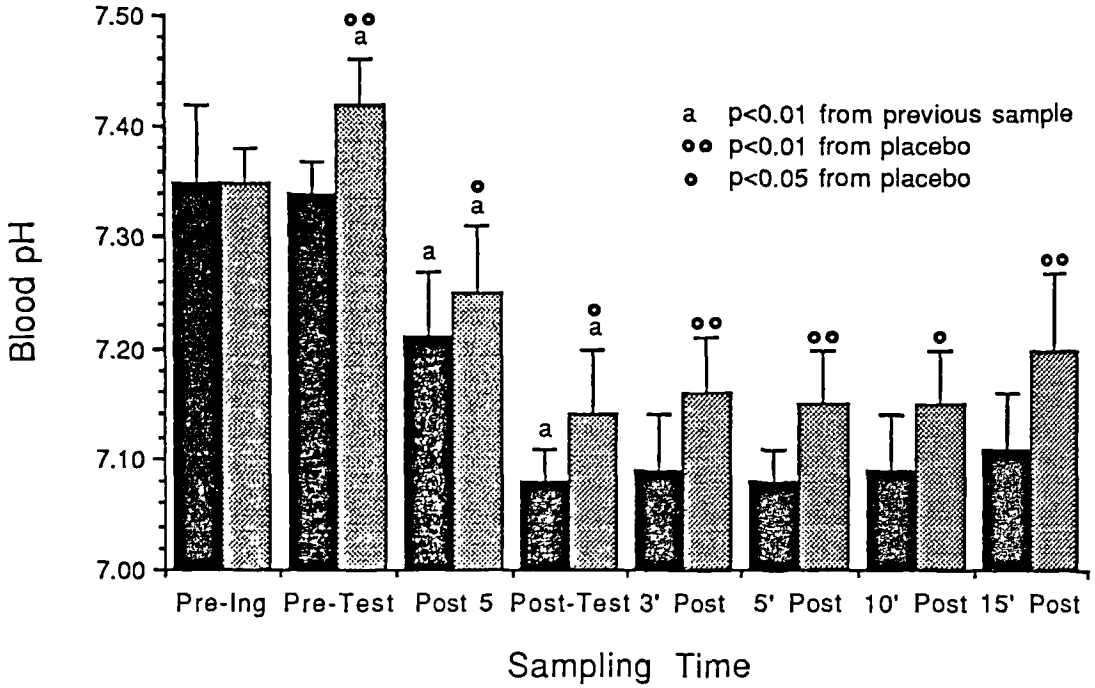


Figure 5.4. Blood pH during exercise and recovery in the placebo and alkaline conditions (mean ± s.d, n=8).

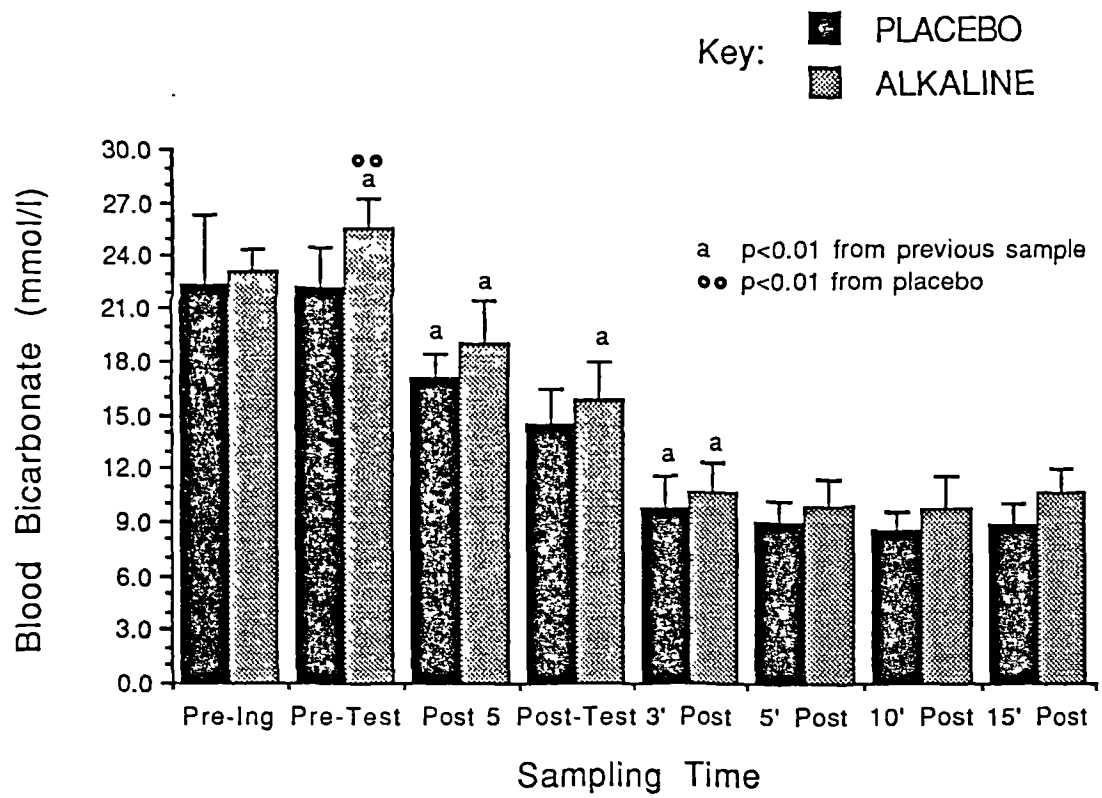


Figure 5.5. Blood bicarbonate concentrations (mmol/l) during exercise and recovery in the placebo and alkaline conditions (mean ± s.d., n=8).

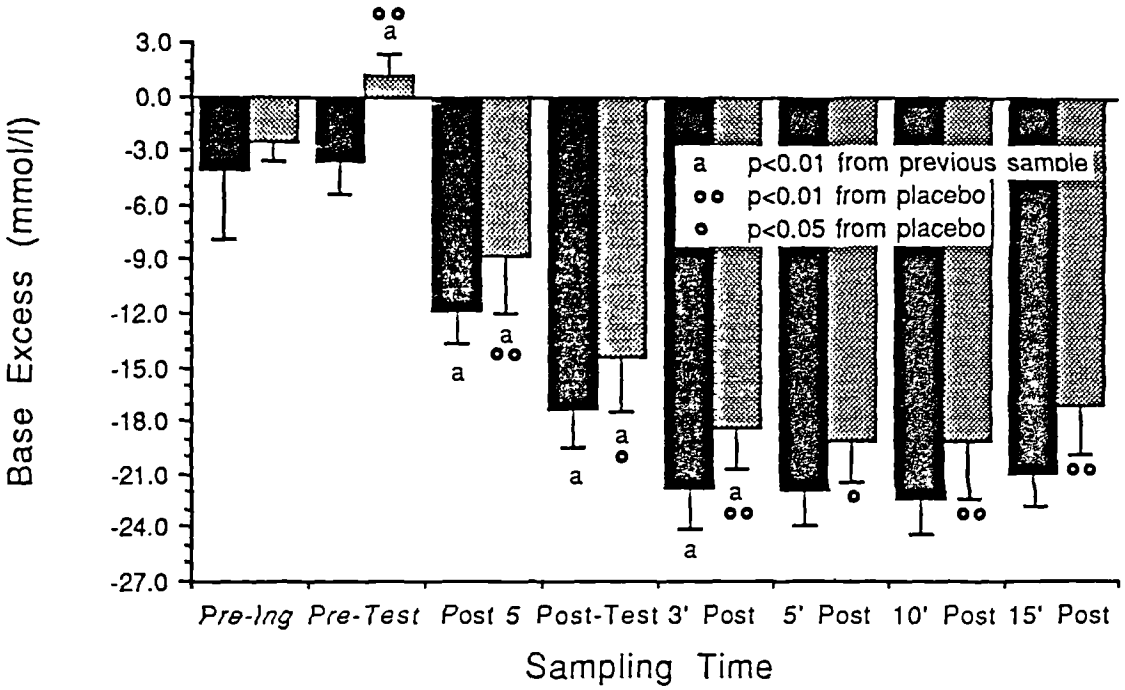


Figure 5.6. Blood base excess concentrations (mmol/l) during exercise and recovery in the placebo and alkaline *conditions* (mean ± s.d., n=8).

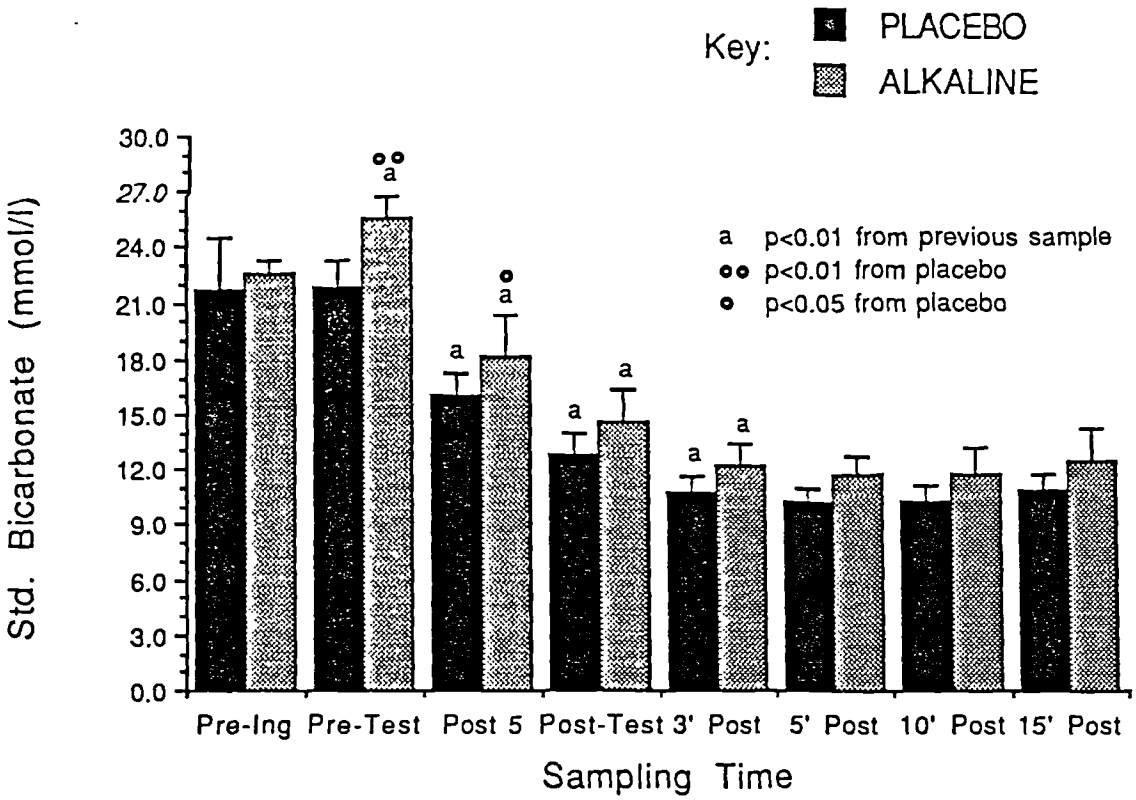


Figure 5.7. Blood standard bicarbonate concentrations (mmo/l) during exercise and recovery in the placebo and alkaline conditions (mean ± s.d., n=8).

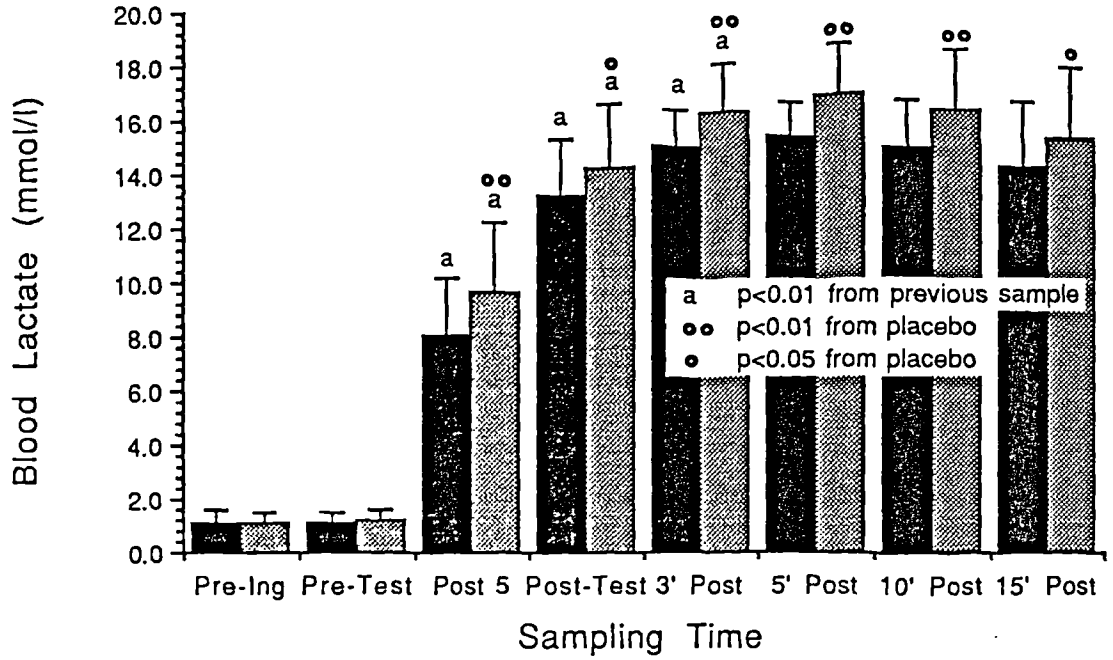


Figure 5.8. Blood lactate concentrations (mmol/l) during exercise and recovery in the placebo and alkaline conditions (mean ± s.d., n=8).

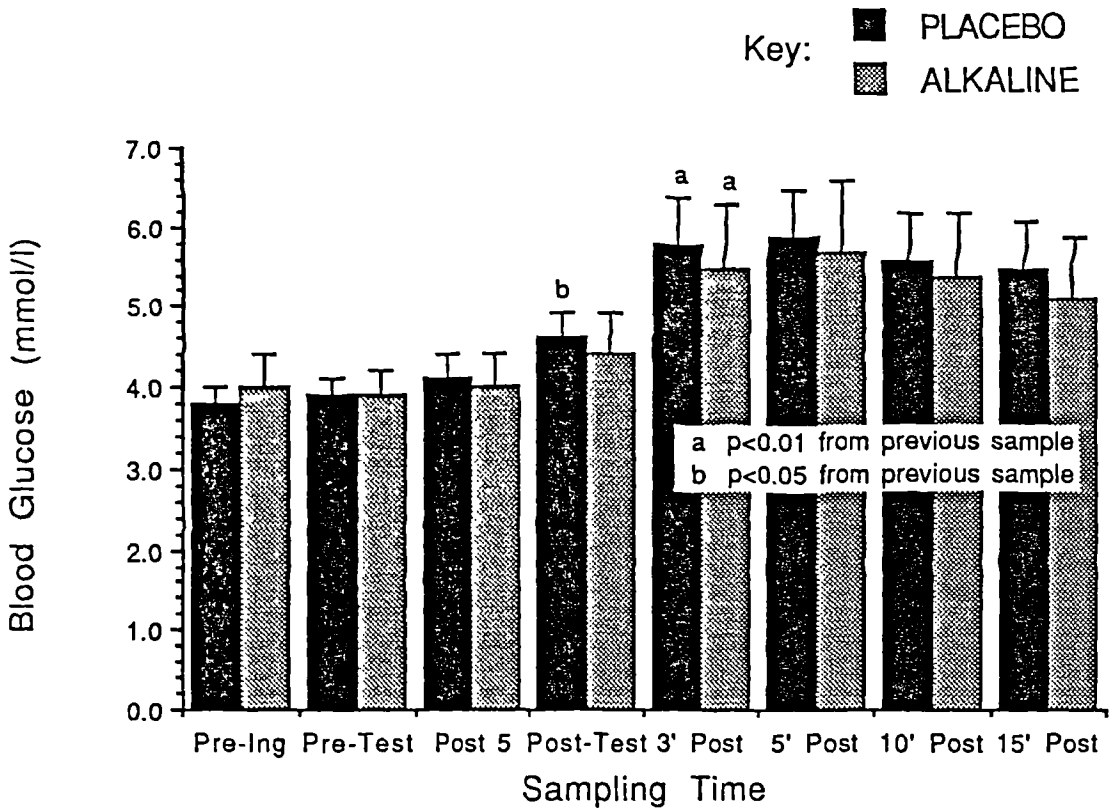


Figure 5.9. Blood glucose concentrations (mmol/l) during exercise and recovery in the placebo and alkaline conditions (mean ± s.d., n=8).

the exercise test. Despite the higher post-exercise blood lactate concentrations following the alkaline treatment, the decrease in blood pH and base excess in all post-exercise samples was similar in both conditions. Blood pH fell to its lowest value immediately after exercise, irrespective of condition, by 0.26 ± 0.05 units in the placebo trial and by 0.28 ± 0.09 units in the alkaline trial (n.s. between conditions). Base excess concentration had decreased by 18.4 ± 1.4mmol.l^{-1} in the placebo and 20.4 ± 2.1mmol.l^{-1} in the alkaline condition, at 5min post-exercise, but the fall was not different between treatments.

With the exception of the pre-exercise value, no differences were found in blood HCO_3^- and standard HCO_3^- concentrations between conditions in all blood samples obtained post-exercise. It was noted however, that the fall in HCO_3^- and standard HCO_3^- concentrations was greater in the alkaline trial compared with the placebo in all samples during recovery ($p<0.05$). Blood HCO_3^- and standard HCO_3^- fell to their lowest value after 5 minutes of recovery, irrespective of condition (HCO_3^-:9.0 ± 1.2mmol.l^{-1} vs 9.9 ± 1.5mmol.l^{-1} and standard HCO_3^-:10.3 ± 0.7mmol.l^{-1} vs 11.7 ± 1.1mmol.l^{-1} in the placebo and alkaline conditions, respectively) and continued to remain that low until the end of the recovery period.

Urine pH

Following alkalisation the formation and excretion of alkaline urine was evident. Resting pre-exercise urine pH was 6.20 ± 0.86 in the placebo and 8.09 ± 0.14 in the alkaline condition ($p<0.01$).

Blood glucose

The responses of blood glucose to the exercise-test are shown in Figure 5.9. No differences were found in blood glucose concentrations between the two conditions at any individual stage. Peak blood glucose concentration generally occurred at 5min post-exercise (5.9 ± 0.6mmol.l^{-1} placebo and 5.7 ± 0.9mmol.l^{-1} alkaline) and was maintained above resting values ($p<0.01$) over the subsequent 10min of recovery.

Plasma catecholamines

The resting plasma AD and NA concentrations were not affected by ingestion of $NaHCO_3$. Both plasma AD and NA concentrations increased several fold above resting values following completion of sprint 5 ($p<0.01$) and reached peak values immediately after exercise, irrespective of condition. Thereafter, with the exception of the 3min post-exercise sample, plasma AD concentrations were no different from resting values (Figure 5.10). In contrast, plasma NA concentrations were higher than at rest ($p<0.01$) for all samples taken during the recovery period following completion of the exercise test (Figure 5.11). In addition, both AD and NA concentrations, immediately after exercise were found to be lower in the alkalotic condition than in the placebo *condition ($p<0.01$) (AD*: $6.5 \pm 1.6 nmol.l^{-1}$ vs $4.7 \pm 1.7 nmol.l^{-1}$ and NA: $31.1 \pm 8.9 nmol.l^{-1}$ vs $25.9 \pm 6.6 nmol.l^{-1}$).

Change in plasma volume

There were considerable increases, above resting values, in blood haemoglobin concentration and haematocrit values immediately after exercise ($p<0.01$), indicating that a *significant fluid shift* had occurred as a result of the exercise test. The greatest percentage decrease in plasma volume generally occurred immediately after exercise, irrespective of condition, and was $14.2 \pm 3.1\%$ and $13.3 \pm 4.9\%$ for the placebo and bicarbonate trials, respectively. The changes in plasma volume were not different between conditions.

Muscle metabolites

The resting, post-sprint 5 and post-exercise muscle metabolite concentrations for the placebo and alkaline conditions are shown in Table 5.2 and Table 5.3, respectively. Following the bicarbonate treatment there were no changes in the resting concentrations of any metabolite compared with placebo. In addition, despite the considerable changes in the concentrations of most metabolites noted after sprint 5 and post-exercise, as a result of the maximal nature of each exercise bout, these changes remained unaffected by prior administration of $NaHCO_3$.

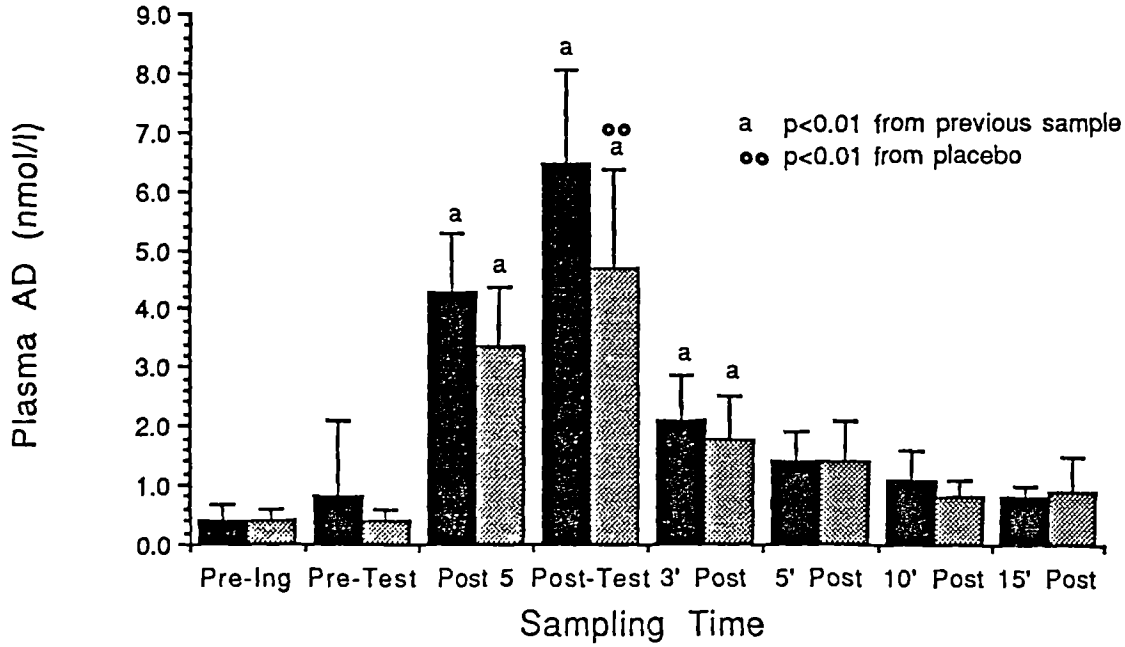


Figure 5.10. Plasma adrenaline concentrations (nmol/l) during exercise and recovery in the placebo and alkaline conditions (mean ± s.d., n=8).

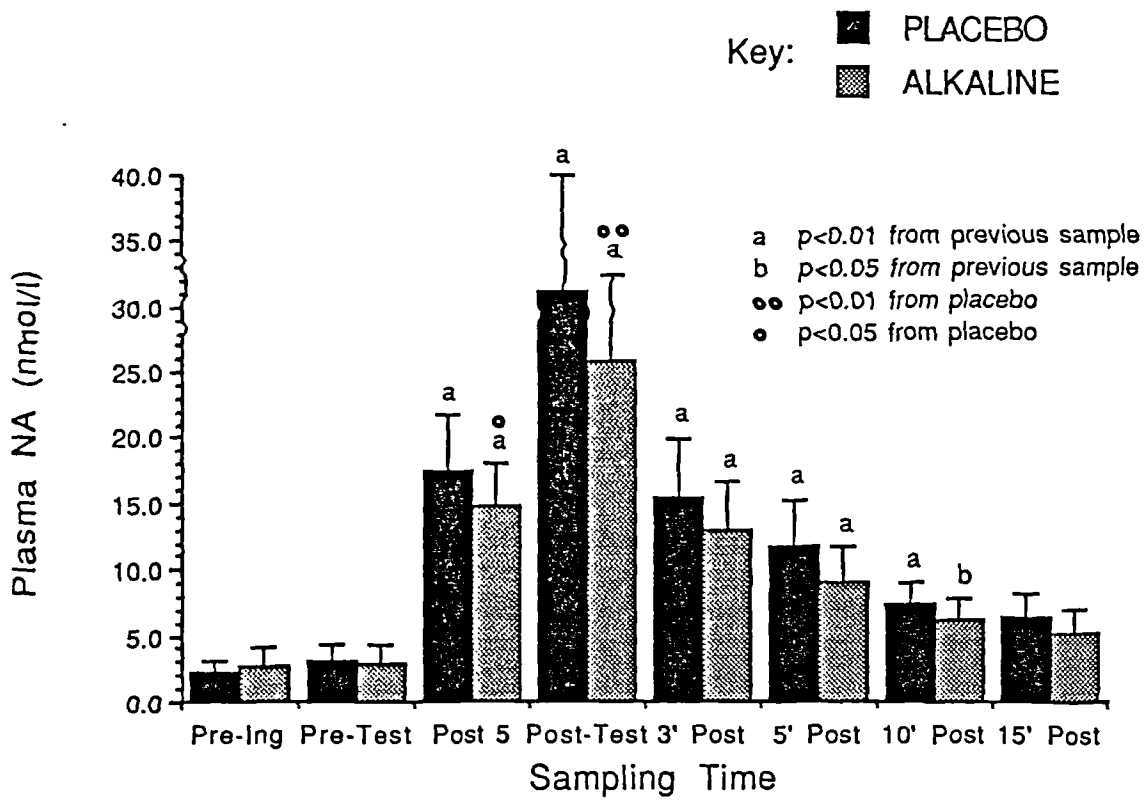


Figure 5.11. Plasma nor-adrenaline concentrations (nmol/l) during exercise and recovery in the placebo and alkaline conditions (mean ± s.d., n=8).

The first five sprints, during which subjects completed the same amount of work, in both conditions, resulted in a similar fall in PCr concentration (63%) for both conditions. Although no changes were observed in ADP and AMP concentrations during the first five sprints, the concentration of ATP decreased by 30% in the placebo and 24% in the alkaline conditions ($p<0.01$) after five sprints. Approximately 27% of the total glycogen pool was mobilised ($p<0.01$), irrespective of condition, which was accompanied by significant increases in muscle G-6-P (15-fold), F-6-P (15-fold), F-1,6diP (5-fold) and lactate (25-fold) concentrations ($p<0.01$). There were relatively modest increases ($p<0.01$) in muscle glucose (4-fold), G-1-P (4-fold) and pyruvate (4 to 5-fold) concentrations, with no change in the concentration of triose phosphates under both conditions.

Following completion of the entire exercise-test the concentration of muscle glycogen was slightly reduced (n.s.) compared with that observed after sprint 5 whereas muscle glucose concentration had increased further ($p<0.01$) in both conditions. Total ATP concentration was reduced at the end of the test by 34% in the placebo and 38% in the alkaline condition from resting values ($p<0.01$). There was no difference in the total decrease in ATP concentration between treatments. The PCr content did decrease further after sprint 5, in both conditions, so that at the end of exercise PCr concentration was 76% below pre-exercise values. There was almost no further change in the concentrations of ADP, AMP, G-1-P, TP and pyruvate after sprint 5 for both conditions. The concentrations of G-6-P and F-6-P were reduced ($p<0.01$) but not below resting levels ($p<0.01$), in contrast to the concentration of F-1,6-diP which had fallen to resting levels. Unlike the decrease in the hexose monophosphates, muscle lactate concentration was further increased after sprint 5 ($p<0.01$), but not affected by the treatments. A higher correlation was observed between F-6-P and F-1,6-diP after sprint 5 in the alkaline condition ($r=0.890$, $p<0.01$) than in the placebo ($r=0.828$, $p<0.05$).

Muscle pH and buffering capacity

Resting muscle pH, as determined by the homogenate technique was not affected following alkalisation (7.11 ± 0.04; placebo vs 7.10 ± 0.03; alkaline). Resting muscle buffering capacity, as determined by HCl-titration on the muscle homogenates, was not altered by prior ingestion of $NaHCO_3$ (24.05 ± 1.83mmol $H^+.kg^{-1}$ wm; placebo vs 22.9 ± 1.18mmol $H^+.kg^{-1}$ wm; alkaline). Muscle pH fell by 0.22 ± 0.09 units in the placebo and 0.18 ± 0.07 units in the alkaline conditions following completion of five sprints and by a further 0.12 ± 0.06 units and 0.13 ± 0.08 units, respectively, at the end of the exercise (Table 5.4). These changes were not statistically significant between treatments. Interestingly, those individuals who had the lowest pH after sprint 5 had the highest muscle lactate ($r=-0.874$, $p<0.01$) in the alkaline condition, and the highest G-6-P concentration ($r=-0.947$, $p<0.01$) in the placebo condition.

TABLE 5.2 Muscle metabolites before exercise and after the fifth and last sprints of the multiple sprint test in the placebo condition (mean ± s.d., muscle glycogen in mmol.glucosyl units.kg^{-1} dm; all other values in mmol.kg^{-1} dm).

METABOLITES	PRE-1st (n=8)	POST-5th (n=8)	POST-TEST (n=8)
Glycogen	335.8±41.3	247.4±60.0**	230.1±61.0**
Ac sol glyc	32.6±14.1	5.2±7.6**	4.0±10.1**
Ac insol gl	303.3±32.7	242.3±53.5**	226.1±53.3**
ATP	22.7±1.4	16.0±1.3**	15.0±2.2**
ADP	3.2±0.5	3.8±0.6	3.7±0.4
AMP	0.1±0.0	0.1±0.1	0.1±0.1
PCr	78.8±7.3	29.0±6.1**	18.4±3.8[a]**
Cr	38.9±8.8	88.7±6.3**	99.4±7.3[a]**
Glucose	1.8±0.5	6.9±1.1**	10.3±2.1[a]**
G-1-P	0.3±0.1	0.9±0.5*	0.8±0.4
G-6-P	1.1±0.9	15.2±3.7**	10.8±4.6[a]**
F-6-P	0.2±0.1	3.3±1.2**	2.5±1.4[b]**
TP	0.4±0.3	0.5±0.3	0.6±0.5
F-1,6-DP	0.5±0.3	2.3±1.3**	0.4±0.2[a]
Lactate	4.1±2.1	89.3±13.6**	128.9±21.3[a]**
Pyruvate	0.5±0.2	2.0±0.4**	1.8±0.6**

a = $p<0.01$ from previous sample
b = $p<0.05$ from previous sample
** = $p<0.01$ from pre-exercise
* = $p<0.05$ from pre-exercise

TABLE 5.3 Muscle metabolites before exercise and after the fifth and last sprints of the multiple sprint test in the alkaline condition (mean ± s.d., muscle glycogen in mmol.glucosyl units.kg^{-1} dm; all other values in mmol.kg^{-1} dm).

METABOLITES	PRE-1st (n=8)	POST-5th (n=8)	POST-TEST (n=8)
Glycogen	345.4±34.8	250.2±65.9**	215.1±49.7**
Ac sol glyc	36.8±11.4	8.9±13.8**	3.6±5.9**
Ac insol gl	308.6±29.9	241.4±53.0**	211.5±44.9**
ATP	22.1±1.9	16.8±3.5*	13.8±1.5**
ADP	4.0±0.3	4.3±0.4	4.4±0.6
AMP	0.2±0.1	0.1±0.1	0.1±0.1
PCr	75.3±7.8	27.9±13.7**	17.8±4.8[a]**
Cr	42.5±10.8	90.5±14.6**	100.1±7.6[b]**
Glucose	1.7±0.7	7.1±1.6**	11.5±2.2[a]**
G-1-P	0.2±0.1	1.0±0.4**	0.7±0.3
G-6-P	1.0±0.5	15.3±3.6**	11.2±3.6[a]**
F-6-P	0.2±0.1	3.1±1.4**	2.0±0.9[a]**
TP	0.4±0.1	0.5±0.1	0.5±0.1
F-1,6-DP	0.4±0.2	2.0±1.2**	0.4±0.1[a]
Lactate	3.0±1.5	85.7±30.4**	130.8±19.1[a]**
Pyruvate	0.4±0.1	2.2±0.6**	2.0±0.4**

a = $p<0.01$ from previous sample
b = $p<0.05$ from previous sample
** = $p<0.01$ from pre-exercise
* = $p<0.05$ from pre-exercise

TABLE 5.4 Muscle pH at rest-15min prior to exercise and immediately after the fifth and last sprints in the placebo and alkaline conditions (mean ± s.d, n=8).

CONDITION	MUSCLE pH		
	PRE-1st	POST-5th	POST-test
Placebo	7.11±0.04	6.89±0.06**	6.77±0.11^{b**}
Alkaline	7.10±0.03	6.92±0.07**	6.79±0.08^{b**}

b = $p<0.05$ from previous sample

** = $p<0.01$ from pre-exercise

5.4 DISCUSSION

In the present study, the amount of work done per sprint was standardised in order to compare the metabolic changes that occur after a given number of sprints and at fatigue, following ingestion of $NaHCO_3$ or NaCl. The power outputs generated by the subjects and the total work accomplished in the first sprint of the exercise-test, irrespective of experimental condition, are similar to those reported in the previous study (Chapter 4) for active male subjects during the first 6s bout of the multiple sprint-test. However, during this type of maximal intermittent exercise, in which subjects perform a given amount of work during each exercise bout, there were more pronounced decrements in power output compared to those observed during repeated sprints of fixed duration (Chapter 4). This is not a surprising finding since the time required to perform the standardised amount of work increased with each subsequent bout of exercise effectively increasing the work to rest ratio. In the present study the recovery period was 30s, irrespective of sprint duration. It has been suggested that for a given recovery period, decrement in performance is not only intensity dependent, but it is also dependent on the duration of the exercise (Lakomy, 1988).

In the previous study and other similar studies which have examined performance during repeated maximal 6s sprints, with 30s recovery between sprints (Holmyard et al, 1988; Brooks et al, 1990), there is no or very little measurable fatigue during the first three sprints as reflected by the decline in PPO and MPO. In contrast, the present study in which exercise was also performed at a maximal intensity and at similar recovery intervals, both PPO and MPO (i.e. work rate) were substantially lower over the second and third sprints in comparison with the first, irrespective of condition. However, the durations of these exercise bouts were on average only 0.4s and 0.8s longer than the first sprint, respectively. Although the decline in MPO is explicable on the basis of a longer time required to achieve the standardised amount of work, it is at present not possible to explain why an increase in the second exercise duration by 0.4s followed by 30s recovery would result in such a pronounced decrement in PPO (approximately 13% in both conditions) in the subsequent exercise bout.

In the present study no significant effect was found on any of the performance variables examined as a result of the ingestion of sodium bicarbonate solution in comparison to the placebo condition. Nevertheless, five out of the eight subjects exercised longer and performed more work under alkalosis. This is because they were capable of completing one more sprint before reaching fatigue (i.e.>10s). Consequently, total time to exhaustion for the whole group was increased by 5% (n.s.) and total work accomplished during this time was greater by 7% (n.s.) compared with placebo condition.

An interpretation of the results in this way however, would seem to be misleading. This is because those subjects that exercised longer in the alkalotic condition did so, by completing their penultimate exercise bout in just under 10s as opposed to just over 10s in the placebo condition and were, therefore allowed to perform one sprint more in the former condition without time restriction. In contrast, by comparing the average rate at which work (J) was performed over unit of time (s), it was evident that the average rate at which subjects were exercising in the alkalotic trial was only 0.8% greater (n.s.) compared to that attained in the placebo condition over the entire test but 3.1% higher (n.s.) between completion of sprint 5 and termination of exercise. Moreover, by excluding the extra single sprint that five subjects performed under alkalosis, the rate at which work was performed by each subject for the same number of sprints, in both conditions, was on average 3.2% greater (n.s.) in the alkaline treatment compared to the placebo.

The results of the present study are not however in agreement with those of previous studies which employed intense but predominantly aerobic exercise. All these studies found considerable improvements in performance as a result of ingesting sodium bicarbonate (Jones et al, 1977; Sutton et al, 1981). In contrast, studies that have involved high-intensity intermittent exercise procedures have provided only inconclusive results. Pre-exercise ingestion of bicarbonate solution has failed to improve maximal power output and total work done over three 30s bouts of maximal cycling (Parry-Billings and MacLarren, 1986). In a recent study however, it was shown that power output during cycling

exercise, consisting of ten 10s sprints with 50s recovery between each sprint, was enhanced following alkalisation (Lavender and Bird, 1989). Similarly, it has been shown that endurance time to exhaustion in the final exercise bout at 125% VO_2 max was significantly increased under alkalotic conditions (Costill et al, 1984), whereas others employing the same exercise protocol found longer endurance time in the alkalotic condition in only three out of the five subjects (Wijnen et al, 1984). These conflicting results were observed despite comparable changes in acid-base balance of the blood as a result of sodium bicarbonate ingestion.

In the present study the buffering capacity of the blood was increased prior to the test during the bicarbonate trial. This was confirmed by the higher ($p<0.01$) pre-exercise HCO_3^-, standard HCO_3^- and base excess concentrations compared with the placebo trial. In addition, pre-exercise pH values under alkalosis were elevated above placebo values by 0.08 units ($p<0.01$). These acid-base changes in the blood were similar to those reported by other authors using strong doses ($0.3g.kg^{-1}$ bw) of sodium bicarbonate (Inbar et al, 1983; McCartney et al, 1983; Wilkes et al, 1983; Parry-Billings and MacLaren, 1986). This dose exceeds the threshold dosage of $NaHCO_3$ necessary to elevate blood bicarbonate (Gao et al, 1988).

Despite the alkalisation of the blood by prior oral administration of sodium bicarbonate, both resting muscle pH and buffering capacity determined by HCl-titration of muscle homogenates (B_{vitro}) were no different from samples obtained under the placebo condition. This could be attributed to the relatively impermeable nature of the sarcolemma to the bicarbonate ion (Robin, 1961). In the present study, therefore, only the extracellular compartment had an increased buffer capacity prior to exercise as a consequence of $NaHCO_3$ ingestion.

Ingestion of bicarbonate does not enhance muscle buffering capacity directly and muscle pH and, yet, it has been shown to improve performance under certain exercise conditions. It would appear that an increased buffering capacity of the blood enhances the efflux of H^+ and lactate ions from the working muscle into the systemic circulation, as

previously suggested (Hirche et al, 1975; Mainwood and Worsley-Brown, 1975). This proposed mechanism is supported by evidence of improved function of isolated frog muscle, in a perfusing medium with a high concentration of HCO_3^- and delayed recovery associated with impaired H^+ efflux from isolated muscle deficient in extracellular HCO_3^- (Mainwood and Worsley-Brown, 1975). If this is the case, then, an increased rate of H^+ would be theoretically advantageous in that the intramuscular pH prior to each sprint would be higher in an alkaline milieu than in the placebo condition. Consequently, a more alkaline environment could affect positively not only the activity of pH dependent enzymes which are considered to be rate limiting for glycolysis (PFK) and glycogenolysis (phosphorylase) (Sahlin, 1978) but also improve contractile activity by reducing the amount of H^+ in the muscle (Fabiato and Fabiato, 1978).

In the present study similar reductions in blood pH, as a result of exercise, but significantly greater decreases in blood standard HCO_3^- and increases in blood lactate concentrations were observed after sprint 5 and following completion of the exercise-test in the alkaline condition. This is in agreement with the idea of greater H^+ and lactate efflux from the working muscle into the circulation during alkalosis. However, similar muscle pH values and lactate concentrations were observed after the first five sprints and at the end of the test, in both experimental conditions. In addition, subjects performed the same total amount of work and the work-rate was the same, irrespective of condition. It would appear therefore, that an enhanced efflux of H^+ from the muscle did occur, as a consequence of the increased buffer capacity of the extracellular fluids, but it was not of sufficient magnitude to change muscle pH.

However, several other mechanisms could also explain the differences in blood lactate concentration between the two conditions. For example, the decreased lactate concentration in blood, in the placebo condition, may be a reflection of increased lactate removal by tissues capable of lactate metabolism. Although lactate uptake (Baron, Iles and Cohen, 1978) and gluconeogenesis (Hems, Ross, Berry and Krebs, 1966) in perfused rat liver has been shown to fall as the pH of the perfusate is

reduced, lactate removal by the kidney is known to increase (Yadkin and Cohen, 1975). Moreover, the decreased lactate concentration in blood, in the placebo condition may also be a reflection of increased lactate oxidation within the muscle. As the oxygen dissociation curve is dependent upon pH, the lower extracellular pH in the placebo condition would suggest increased availability of oxygen to all tissues. Indeed, a linear inverse relationship has been observed between oxygen uptake and H^+ concentration in resting human subjects (Karetzky and Cain, 1970). However, the results of previous studies indicate that no such relationship exists during high-intensity exercise although, a tendency for the subjects to use less oxygen after alkaline treatment compared with control has been reported (McCartney et al, 1983b; Costill et al, 1984; Kowalchuck et al, 1984). In the present study oxygen uptake was not measured but the difference in blood pH between placebo and alkaline was similar to those found in previous studies (McCartney et al, 1983b; Costill et al, 1984; Kowalchuck et al, 1984). It is possible, that the differences in blood pH seen in this study could induce significant changes in oxygen uptake between treatments during exercise and recovery intervals, to account for increased oxidation of lactate in the placebo condition. Similarly, however, it could be argued that there was a greater contribution from aerobic metabolism to the total energy demand in the placebo condition. This in effect would mean that despite the similar muscle lactate content observed in both treatments, the rate of anaerobic glycolysis in the alkaline condition was slightly greater than placebo, resulting in the higher blood lactate concentration observed due to a greater efflux of lactate under alkalosis. Alternatively, the lower adrenaline concentrations that were observed in the alkaline condition (even though they only reach statistical significance after exercise) could exert less of a glycogenolytic stimulation compared with placebo. On this basis it could be suggested that lactate concentration should be lower during the alkalosis trial. However, this was not the case, further supporting an increased efflux of H^+ and lactate from muscle to blood.

It would appear therefore, that if an improved efflux of H^+ did occur in the alkalotic condition, it was not of sufficient magnitude to significantly affect the activity of key glycolytic enzymes.

Consequently, the rate of energy production was not affected, as indicated by the concentration of the muscle metabolites after sprint 5 and after completion of the exercise test.

In contrast, previous studies that have examined muscle concentrations of lactate and glycolytic intermediates following alkalisation during predominantly aerobic exercise have concluded that both a greater rate of glycolysis and an enhanced rate of H^+ efflux contributed to a longer endurance time compared with normal or acidotic conditions (Sutton et al, 1981). All improvements in performance during high-intensity exercise after alkalosis, have been shown with exercise durations of 2min or more. Therefore, it would seem that the increased buffering capacity of the blood requires time to facilitate H^+ efflux. The importance of exercise time has also been stressed in previous studies involving an intermittent exercise. Costill and co-workers (1984) showed a 42% improvement in endurance time during the fifth bout of exercise at 125% VO_2 max. It could be expected therefore, that the 30s recovery intervals in the present study would favour efflux of H^+ between each sprint. If this condition occurred, then significantly higher glycolytic rates could be maintained in the subsequent exercise bouts in the alkaline condition. However, this does not appear to have occurred in the present study.

One possible explanation for the conflicting findings of the present study compared to those previously reported, may be that the rate of H^+ and lactate efflux are near maximal during such high-intensity exercise. Thus, reducing the potential benefit of an increased buffering capacity of the blood during this type of exercise. It has also been shown previously, that the diffusion rate of lactate increases linearly with the muscle lactate content up to a muscle lactate concentration of approximately 20mmol.kg^{-1}dm (Jorfeldt et al, 1978). A further increase of muscle lactate showed no further increase in lactate efflux indicating a possible saturation of a lactate translocation process or a maximal rate of lactate release. Alternatively, it may be that the increased buffering capacity of the blood, as a result of sodium bicarbonate ingestion, was inadequate to make any significant difference to the rate of H^+ accumulation because the rates of H^+ production were

too great. Thus, it could not improve the concentration gradient sufficient to increase efflux. In contrast, the greater H^+ efflux observed in isolated animal preparations may be attributable to much greater increases in extracellular buffer concentrations than can be attained using oral alkalisers in man (Mainwood and Worsley-Brown, 1975). In addition, it may also be that the increase in muscle water content during exercise, may have restricted lactate diffusion into the circulation (Bergstrom et al, 1971). The percentage decrease in plasma volume averaged 14.2 $\pm$ 3.1% in the placebo condition and 13.3 $\pm$ 4.9% in the alkaline condition. These are marked changes considering that subjects only exercised for an average of 86s and 90s, respectively.

It is also possible that despite an improved H^+ efflux under alkalosis, muscle pH prior to each sprint remained at a similar low level in both conditions as a result of H^+ release due to resynthesis of PCr. Assuming an average rate of PCr resynthesis of 1.0mmol.kg^{-1}dm.s^{-1} (1.5mmol.kg^{-1}dm.s^{-1} study 1, Chapter 4 and 0.5mmol.kg^{-1}dm.s^{-1}, Ren et al, 1988), during the 30s of recovery (ΔPCr=30mmol.kg^{-1}dm), this would correspond to a release of approximately 15mmol H^+.kg^{-1}dm at a muscle pH 6.8 [0.5 mol H^+ will be released per mol PCr, (Hultman and Sahlin, 1980)].

It could also be argued that this type of intermittent exercise employed in the present study does not stress anaerobic glycolysis to its limits. In contrast, in previous studies during continuous or intermittent exercise of similar duration but of lower work-rate, in which an improvement in performance was shown under alkalosis, anaerobic glycolysis was probably making a more significant contribution to ATP resynthesis. Therefore, a reduced glycolytic flux may have been the principle cause of fatigue in those studies. However, it was shown in Chapter 4 (study 1), that anaerobic degradation of glycogen to lactate formation is maximally engaged during repetitive brief maximal exercise. Moreover, the high muscle lactate concentrations observed in the present study following completion of sprint 5 and at fatigue, further support the idea of a considerable contribution of anaerobic glycolysis to ATP production during this type of maximal exercise. It was also evident in the present study that the concentration of all glycolytic intermediates

was lower at exhaustion compared with sprint 5, suggesting that glycogenolysis was inhibited presumably at the flux-generating step controlled by phosphorylase. In addition, the proportionally larger accumulation in muscle G-6-P and F-6-P relative to F-1,6-diP concentration at fatigue, in comparison with that observed following completion of sprint 5, would suggest that PFK activity was also reduced at the latter stages of the test. It would appear therefore, that the decline in power output in the present study was accompanied by a reduced glycolytic flux, irrespective of condition.

In explaining the fatigue seen in the present study, it is open to speculation whether a decrease in muscle pH to approximately 6.8 in both conditions was responsible for the dramatic reduction observed in power output (45%). Similar muscle pH values have been observed at fatigue during continuous maximal dynamic exercise (Nevill et al, 1989). In contrast, in studies of intense but not maximal exercise muscle pH values are considerably lower at fatigue (Sharp et al, 1986; Bell and Wenger, 1988) or exhaustion (Hermansen and Osnes, 1972). Moreover, force production with electrical stimulation at 20Hz is only reduced by 12.4% after 25.6s of stimulation and muscle pH is decreased to 6.7 (Spriet et al, 1987b), a value only slightly lower than that measured in the present study. It would appear therefore, that in maximal dynamic exercise during which high rates of energy production are required, accumulation of by-products other than H^+, such as Pi, must be greater, which may contribute to fatigue. This may partly explain why there were no significant differences between treatments in the present study.

The high muscle lactate concentrations found immediately after exercise, irrespective of condition, are associated with greater decreases in muscle pH (Hultman and Sahlin, 1980) than those measured in the present study at fatigue. It is possible however, that during the recovery periods of the exercise-test, the removal of lactate and H^+ from the muscle did not necessarily follow the same time course, with the rate of H^+ efflux exceeding that of lactate. Benade and Heisler (1978) found a greater efflux of H^+ than of lactate from stimulated isolated rat diaphragm and frog sartorius muscle. Although the significance of this in vitro study is not clear in an in vivo situation with intact muscle

blood flow, several other authors (Osnes and Hermansen, 1972; Sahlin et al, 1978; Medbo and Sejersted, 1985) have observed that the total acid load seems to exceed the lactate accumulation in blood. This dissociation in the efflux kinetics of H^+ and lactate ions is supported in the present study, irrespective of condition, by the larger decrease in blood base excess (Figure 5.6) compared with the increase in blood lactate (Figure 5.8) provided that the distribution volume for the two ions is the same.

Further evidence that H^+ efflux was greater than the lactate anion could possibly be provided by estimating muscle buffering capacity from the changes in muscle lactate concentration and muscle pH values observed at fatigue (B_{vivo}). In contrast to B_{vitro} which is considered to measure physicochemical buffering only, B_{vivo} also includes H^+ uptake by the metabolic processes and possibly, the unequal efflux of H^+ and lactate anion from the muscle (Hultman and Sahlin, 1980). In vivo muscle buffer capacity in the present study was calculated to be 85.4mmol $H^+.kg^{-1}$ $wm.pH^{-1}$ in the placebo and 99.1mmol $H^+.kg^{-1}$ $wm.pH^{-1}$ in the alkaline condition. These values are approximately 3-4 times higher than those found determined by HCl titration of the muscle homogenates over the pH range 7.1-6.5 (24mmol $H^+.kg^{-1}$ $wm.pH^{-1}$ in placebo and 22.9mmol $H^+.kg^{-1}$ $wm.pH^{-1}$ in the alkaline). Whether this higher muscle buffering capacity, as determined by calculation, does indicate, in addition to buffering by metabolic processes, a greater H^+ efflux or reflects contamination of the post-exercise biopsy muscle sample with blood is open to interpretation.

Although exercise intensity, duration of exercise and/or the total amount of work completed have all been related to increases in catecholamine concentration during prolonged or graded exercise (Galbo, 1983; Lehman, Kapp, Himmelsbach and Keul, 1983; Kjaer, Christensen, Sonne, Richter and Galbo, 1985; Jezova et al, 1985), the factors influencing the magnitude of the catecholamine response to brief maximal exercise are still not known. In the present study, following alkalisation subjects had completed on average more work, they had exercised longer but the rate at which the work was performed was almost

equal to that attained in the placebo condition. Still, the magnitude of plasma catecholamine concentrations was considerably reduced when compared with placebo condition.

The reason for the lower concentrations of both plasma adrenaline and nor-adrenaline under alkalotic conditions in the present study, is not clear but may indicate that the overall sympathetic activity was reduced compared with placebo condition. Similar findings have been reported in a recent study which showed that the rise in adrenaline and nor-adrenaline concentrations from rest to exhaustion (75.3s, 125% VO_2 max) was significantly less under alkalotic conditions (AD:34% and NA:30%) compared with control (Bouissou, Defer, Guezennec, Estrade and Serrurier, 1988). It was suggested in that study, that the reduced catecholamine response to high-intensity exercise in alkalosis was possibly mediated by changes in local factors that depend upon acid-base alterations within the muscle cell rather than a reduced central command from the rostral brain. Further support of these suggestions is evident from studies that have recorded muscle sympathetic activity with microelectrodes in the peroneal nerve during both static and rhythmic handgrip (Victor, Bertocci, Pryor and Nunnaly, 1988). It was shown that the onset of sympathetic activation in resting muscle coincided with the development of cellular acidification in contracting muscle. Increases in muscle sympathetic activity were shown to be closely related, both in time course as well as magnitude with intracellular pH as determined by ^{31}P-NMR but dissociated from changes in local metabolites such as PCr, Pi and ADP (Victor et al, 1988). Owing to this close relationship, between intracellular pH and muscle sympathetic activation it was concluded that extracellular muscle afferent receptors were either directly activated by cellular efflux of H^+ or stimulated by some other metabolites whose cellular transport is pH dependent.

In the present study the reduced concentration of plasma catecholamines in alkalosis does not appear to be explicable on the basis of a different intracellular metabolic environment, as muscle pH and the concentration of muscle metabolites measured after sprint 5 and immediately post-exercise were similar between conditions. It is possible however, that owing to an increased alkalisation in the

extracellular space, fluid surrounding muscle fibres was less acidic in the alkalotic condition, despite an apparent increased efflux of H^+ from muscle compared with the placebo condition. This is also supported by the higher pH values observed in venous blood during and after exercise in the alkalotic condition. If this is the case, then it would seem that the lower extracellular pH in the placebo condition elicited an increase in catecholamine response to exercise in accordance with previous suggestions (Longhurst and Zelis, 1979; Victor et al, 1988). These observations may explain the high negative correlations between blood pH and plasma adrenaline concentrations that have been reported following short-term maximal continuous exercise (Cheetham et al, 1986; Brooks et al, 1988; Nevill et al, 1989).

The possibility however, that this difference in plasma adrenaline and nor-adrenaline concentrations in placebo trial may reflect a decreased metabolic clearance cannot be dismissed. Plasma catecholamines are eliminated in various tissues (such as liver, gut and kidney) by non-neural mechanisms or by uptake in sympathetic noradrenergic nerves the function of which, might have been influenced by a low blood pH. Moreover, whether changes in blood osmolarity as a result of $NaHCO_3$ ingestion may have influenced central control of sympathetic activity is not known. No differences however, were noted between the percentage decrease in plasma volume under the two conditions.

Despite the high circulating levels of plasma adrenaline in the placebo condition, the degree of hyperglycaemia in venous blood was not different between experimental conditions. It has been proposed that liver glycogenolysis is responsible for the post-exercise increase in blood glucose after short-term high-intensity exercise (Lavoie, Bonneau, Roy, Brisson and Helie, 1987). It is likely that other factors, in addition to a high concentration of adrenaline, may also stimulate liver glycogenolysis. Indeed an increase in pancreatic glucagon production has been demonstrated during maximal intermittent exercise (Naveri et al, 1985).

In summary, the results of the present study suggest that the bicarbonate treatment failed to delay the onset of fatigue during repetitive brief maximal exercise in comparison with the placebo treatment. Although, alkali ingestion resulted in significant shifts in acid-base balance of the blood, it had no effect on muscle metabolism during this type of exercise. There was, nevertheless, a reduction in the catecholamine response to exercise during the alkalotic condition compared to the placebo condition.

6. THE EFFECT OF CIRCULATORY OCCLUSION ON PERFORMANCE DURING INTERMITTENT MAXIMAL EXERCISE

6.1 INTRODUCTION

During maximal exercise of short duration the mechanisms and site of human muscle fatigue remain unclear. In study 1 (Chapter 4) skeletal muscle energy metabolism during brief intermittent maximal exercise was examined. During this type of exercise the ATP production required for muscular contraction was dependent mainly on anaerobic pathways such as PCr breakdown and glycolysis leading to lactate formation. Although, the relative contribution made by these processes throughout the exercise period was not fully determined, the study indicated that at the end of exercise the glycolytic rate was considerably reduced and anaerobic energy production was derived predominantly from PCr degradation.

The results of study 2 (Chapter 5) indicated that the rate at which intermittent maximal exercise was performed, was not significantly affected by prior alkalisation of the blood. In addition, the considerable fatigue induced decrements in performance that were observed during this type of intermittent maximal exercise, were not associated with a low muscle pH. It has been shown that during exercise of lower intensity, to exhaustion, muscle pH values are lower than recorded in studies using maximal exercise intensity (Sahlin et al, 1976; Nevill et al, 1989).

A limitation to ATP production is the availability of PCr. Previous studies have shown that the rate of PCr resynthesis may be limited by the availability of oxygen and the restoration of muscle pH (Harris et al, 1976; Sahlin et al 1979). The possibility, therefore, that a reduction in PCr content may be more important contributory factor, than pH, to fatigue, during intermittent maximal exercise, still exists. Therefore, the aim of the present study was to examine performance during maximal exercise whilst occluding the circulation to the thighs

during each recovery interval, with a view to providing circumstantial evidence for the important role of PCr in energy production during exercise.

6.2 METHOD

Subjects

Thirteen healthy male physical education students whose mean (± s.d.) age, height and weight were 24.7 ± 4.2 years, 178.2 ± 6.8cm and 75.0 ± 10.3kg, respectively, volunteered to take part in this study. All experimental procedures were explained to the subjects and a written informed consent was obtained from each subject before the start of the study.

Protocol

Four hours after the last meal, subjects were required to perform ten maximal sprints of 6s duration each, separated by 30s recovery periods, on a cycle ergometer on three separate occasions. On one occasion (occlusion) the blood circulation to both legs was occluded during each recovery interval. This was achieved using two 15cm-wide pneumatic cuffs placed around the proximal portion of the thighs and inflated to a pressure of 270mmHg. The cuffs were connected to a bottle of compressed air (2000 bar) through a gas cylinder regulator (Cryoservises Ltd). In this way it was possible to inflate them to the required pressure within 2-3s following completion of each exercise bout. Three seconds prior to the subsequent exercise bout the cuffs were deflated by disconnecting them from the air supply. Blood circulation to the legs was not occluded during the 10min recovery period following completion of the tenth sprint. On a separate occasion (combined) the cuffs were inflated only during the first six recovery intervals subsequent to their respective sprints, after which the exercise-test was continued with intact circulation. Finally, the control condition was performed with intact circulation throughout the exercise period. The order of testing was randomly assigned.

The power output during each sprint was recorded, whilst subjects were cycling at maximal speed. In addition heart rate was monitored throughout the exercise period and for the first 15min of a passive recovery following exercise.

Blood Sampling

Venous blood samples were taken at rest, after sprint 7, immediately post-exercise and at 3min, 5min and 10min of post-exercise recovery when subjects returned to a recumbent position on an adjacent examination couch. The samples were analysed for blood pH, lactate and plasma ammonia, catecholamines, sodium and potassium concentrations.

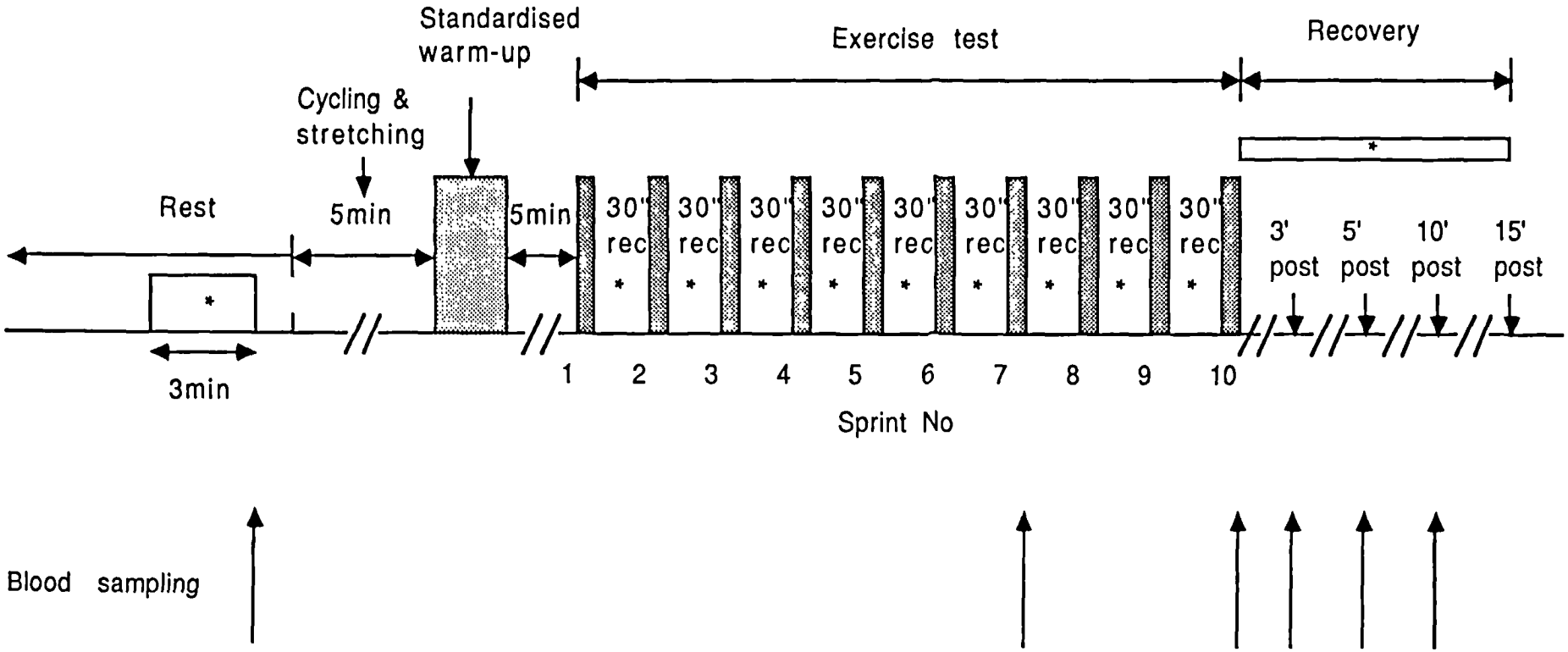
Expired air collection

Samples of expired air were collected at rest during the last 3min of a 5min recovery with the subject in a recumbent position, during the 30s recovery interval subsequent to each exercise bout and for the whole of the 15min post-exercise recovery when the subjects returned to the recumbent position (Figure 6.1). Volume and percentage oxygen of expired air were measured using equipment previously described (see Chapter 3, for methods) from which ventilation and oxygen uptake were determined. At the time of each analysis prevailing room temperature and pressure were recorded and all variables were later converted to STPD.

Blood and expired air samples were taken in the control and occlusion conditions only.

Statistical Analysis

Differences in performance characteristics between any two experimental conditions were examined using a two-way analysis of variance for correlated means with repeated measures on both factors (i.e. sprint number and experimental condition). The same statistical test was used to examine the differences in the metabolic and respiratory responses to the exercise-test between the control and occlusion conditions. A Pearson product moment correlation test was used to examine the relationship between variables. Results are presented as means $\pm$ s.d. Statistical significance was accepted at the 5% level.



* denotes expired air samples

Figure 6.1. Schematic illustration of study's protocol.

6.3 RESULTS

Power output

The mean power (MPO), peak power (PPO) and end power (EPO) outputs generated by the subjects over the ten 6s sprints in all three experimental conditions are shown in Figures 6.2, 6.3 and 6.4, respectively. The highest power outputs were achieved in the first sprint, irrespective of condition. These values were not different between conditions during this initial exercise bout. As the test progressed, significant differences ($p<0.01$) in MPO, PPO and EPO were found between sprints, irrespective of condition, indicating that the cumulative effects of successive sprints resulted in considerable fatigue induced decrements in performance. It was evident however, that when the circulation to the legs was occluded, during each recovery interval, the decline in power output was more pronounced in comparison to the control condition. In the seventh sprint, MPO had fallen by 43.5% in the occlusion condition and 15.8% in the control condition ($p<0.01$), whereas during the final sprint MPO had decreased by 47% and 19.4% of the maximal value ($p<0.01$), in the occlusion and control experiments, respectively. Similar differences between the occlusion and control conditions were also noted in the decline of PPO and EPO. The decrease in PPO after ten sprints averaged 55% and 24.5% ($p<0.01$) for the occlusion and control conditions, respectively, whereas the fall in EPO in the last bout of exercise was 43% and 24% ($p<0.01$), respectively. The average work rate at which subjects were exercising over the ten 6s sprints was 611.2$\pm$ 88.7W in the occlusion and 791.7 $\pm$ 106.4W in the control condition ($p<0.01$).

In the third experimental condition, restoration of the circulation to the thighs following completion of sprint seven, resulted in a considerable improvement in performance over the subsequent exercise bout, despite the short duration recovery period (30s). Mean power output was rapidly restored from 58.5% in sprint 7 to 75.2% of the maximal value in sprint 8 ($p<0.01$). During the final exercise bout MPO had reached 80% of the maximal value. Similar changes were also noted in PPO and EPO. Peak power increased from 49.8% in sprint 7 to 70% of the maximal value in sprint 10 and EPO from 63.6% to 80% of the maximal

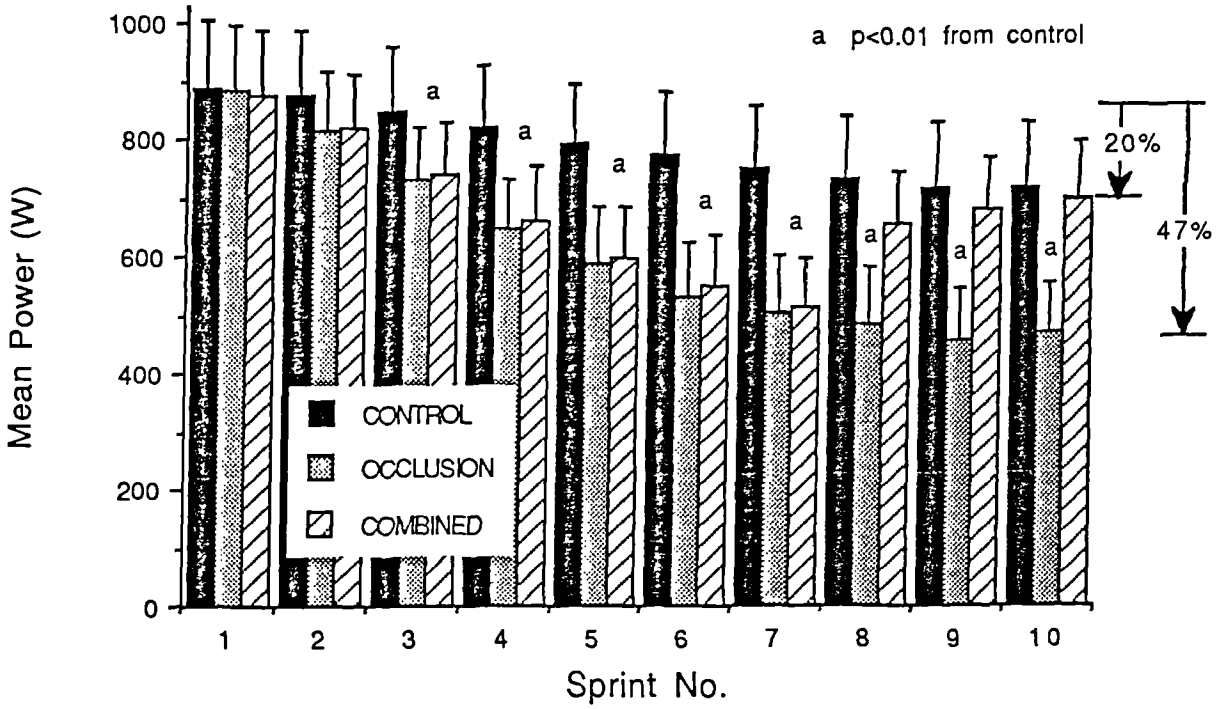


Figure 6.2. Mean power output (W) for each of the ten 6s sprints in all three experimental conditions (mean ± s.d., n=13).

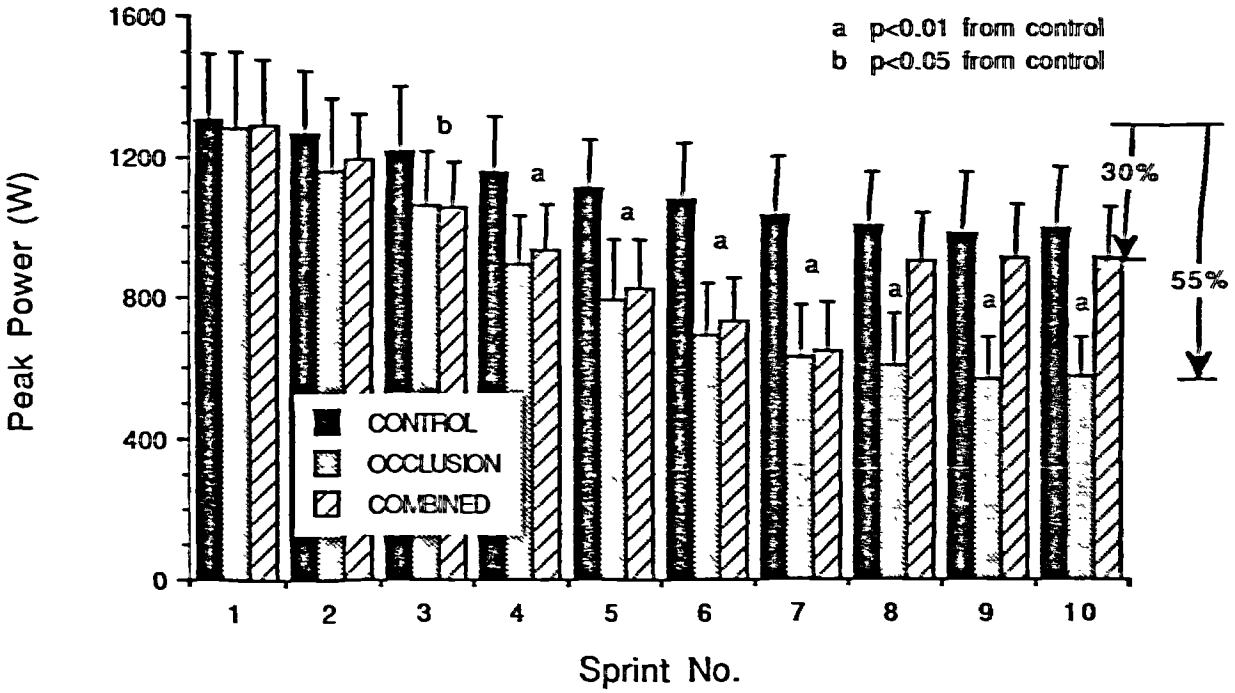


Figure 6.3. Peak power output (W) for each of the ten 6s sprints in all three experimental conditions (mean ± s.d., n=13).

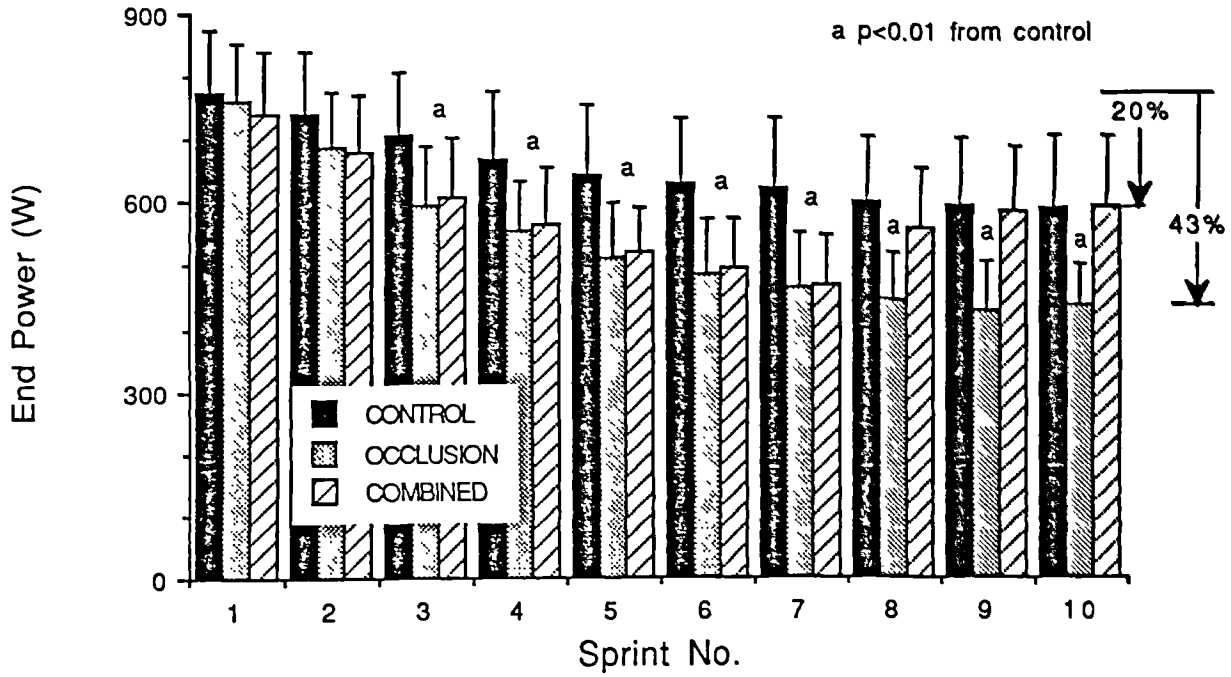


Figure 6.4. End power output (W) for each of the ten 6s sprints in all three experimental conditions (mean ± s.d., n=13).

value, respectively. It is of interest to note, that over the course of the last three exercise bouts following restoration of the circulation to the legs, no statistically significant difference in MPO, PPO and EPO was found at any sprint in comparison to the control condition.

Blood lactate and pH

The blood lactate responses to the multiple sprint-test over the control and occlusion conditions are shown in Figure 6.5. A significant interaction ($p<0.01$) between sampling time and experimental condition was evident. This combined effect indicates that although blood lactate concentrations increased markedly during exercise and recovery following completion of the exercise-test, these increases were altered by occluding the circulation to the legs during the recovery intervals between sprints. Blood lactate concentration at rest was similar between conditions but following completion of seven and ten sprints with the circulation occluded, values were lower compared with control ($6.3 \pm 1.5 mmol.l^{-1}$ vs $7.5 \pm 1.1 mmol.l^{-1}$, $p<0.05$, sprint 7 and $7.8 \pm 1.8 mmol.l^{-1}$ vs $9.4 \pm 0.8 mmol.l^{-1}$, $p<0.01$, sprint 10, respectively). Blood lactate concentration was highest after 5min of recovery, irrespective of condition, and there were no differences in lactate concentrations between trials ($10.0 \pm 1.9 mmol.l^{-1}$ in occlusion and $10.7 \pm 1.2 mmol.l^{-1}$ in the control). Ten minutes after cessation of exercise, blood lactate was still elevated above pre-exercise values ($p<0.01$) in both trials.

Blood pH was similar at rest between conditions but fell from 7.37 ± 0.02 to 7.2 ± 0.04 in the control and from 7.37 ± 0.01 to 7.26 ± 0.05 in the occlusion condition following completion of sprint 7 ($p<0.01$). Blood pH reached its lowest value immediately after exercise which was 7.17 ± 0.04 in the control and 7.24 ± 0.06 in the occlusion condition ($p<0.01$). Blood pH remained that in the occlusion condition during the subsequent 5min of recovery, whereas it had recovered to 7.22 ± 0.04 in the control condition ($p<0.01$). After 10min of recovery blood pH was still lower ($p<0.01$) than resting values (Figure 6.6). Higher correlations were found between blood lactate concentrations and blood

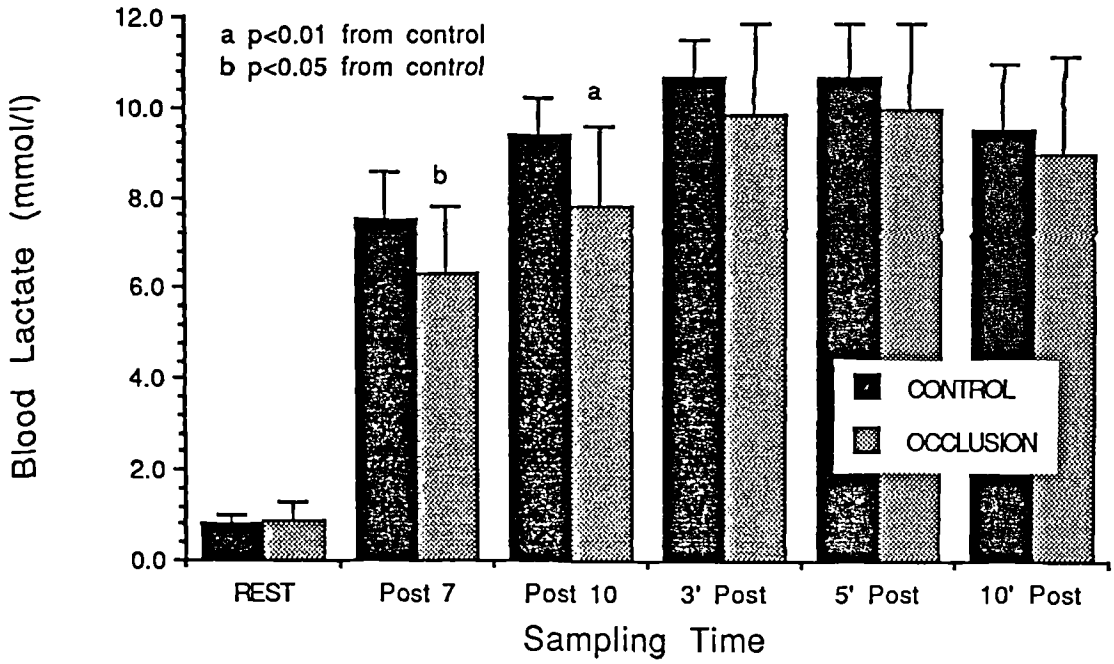


Figure 6.5. Blood lactate concentrations (mmol/l) during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

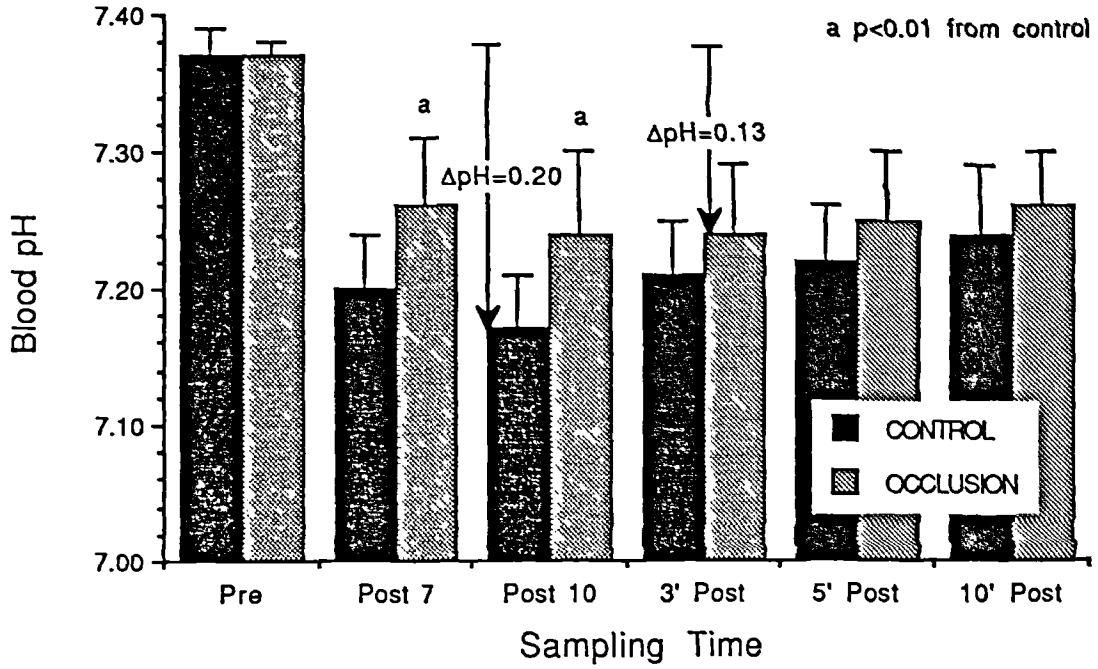


Figure 6.6. Blood pH during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

pH during exercise and recovery in the occlusion compared with the control condition (e.g. post-exercise sample r=-0.952, p<0.01 vs r=-0.724, p<0.01). The average work rate (average MPO) attained over the entire sprint-test in the occlusion condition was correlated with both the blood pH (r=-0.849, p<0.01) and 3min post-exercise blood lactate concentration (r=0.756, p<0.01). This relationship was not as strong for the control condition (r=-0.360 and r=0.190, respectively).

Plasma ammonia

Plasma ammonia concentration at rest was 43.6 ± 25umol.l^{-1} in the control and 39.5 ± 22.2µmol.l^{-1} in the occlusion condition (n.s.). After seven sprints plasma ammonia was higher than resting values (p<0.01), irrespective of condition. Following the completion of the exercise test it reached values of 112.6 ± 21.8µmol.l^{-1} in the control and 82.6 ± 30.4µmol.l^{-1} in the occlusion condition (p<0.05). Plasma ammonia concentration was highest after 5min of recovery in both experimental conditions (184.2 ± 32.6µmol.l^{-1}; control and 172.4 ± 43.4µmol.l^{-1}; occlusion) and remained elevated in the subsequent five minutes of recovery (Figure 6.7). In the occlusion condition, plasma ammonia concentrations were correlated with blood lactate concentrations and *blood pH values (e.g. post-exercise sample r=0.800, p<0.01 and* r=-0.737, p<0.01, respectively). In addition, the increase in plasma ammonia concentration after ten sprints with the circulation to the legs occluded in the recovery intervals, was also correlated with the average MPO attained in that condition over the entire exercise-test (r=0.779, p<0.01).

Plasma catecholamines

Plasma adrenaline concentration increased in response to the first seven sprints; there was a 13-fold increase from 0.3 ± 0.1nmol.l^{-1} to 3.8 ± 0.8nmol.l^{-1} in the control and a 18-fold increase from 0.3 ± 0.1nmol.l^{-1} to 5.4 ± 1.5nmol.l^{-1} in the occlusion condition (p<0.01). The peak plasma adrenaline concentration occurred later in the control in comparison with the occlusion condition (sprint 10 vs sprint 7). Plasma adrenaline concentration was not different from resting after 10min of recovery, irrespective of condition (Figure 6.8).

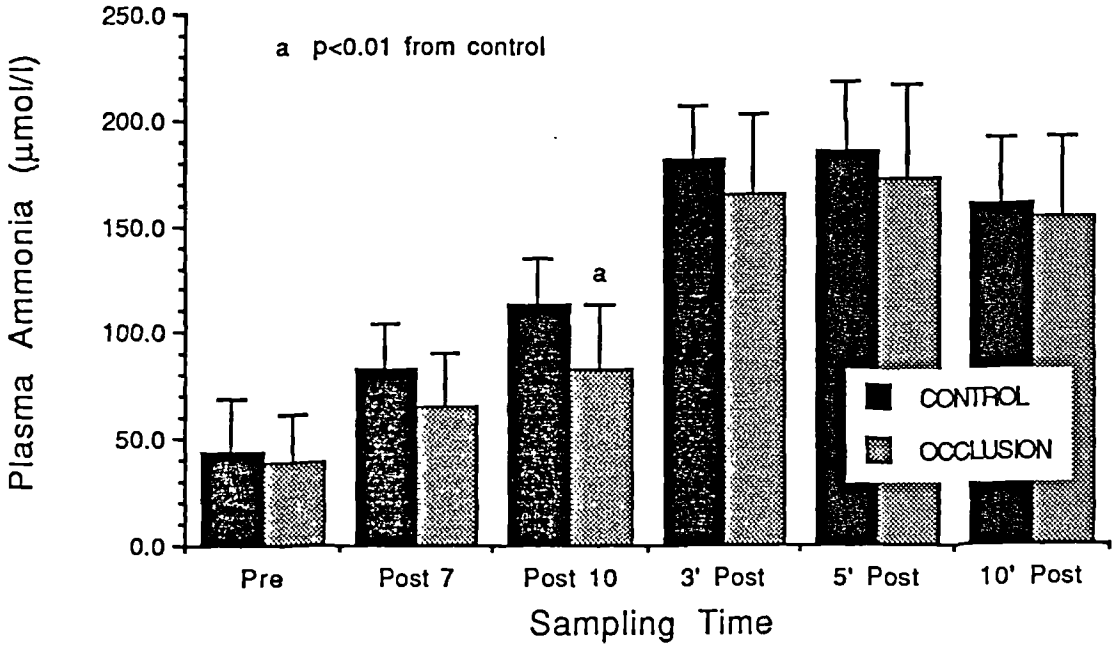


Figure 6.7. Plasma ammonia concentrations (µmol/l) during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

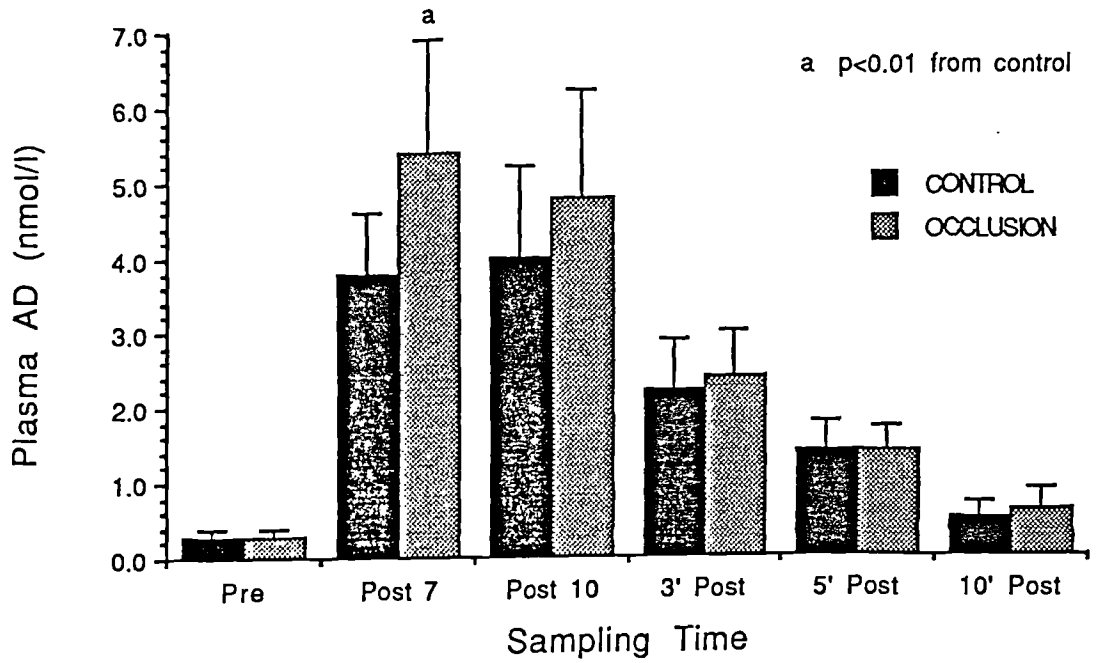


Figure 6.8. Plasma adrenaline concentrations (nmol/l) during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

Plasma nor-adrenaline concentration was higher in the control compared with the occlusion condition after seven sprints (19.2 ± 6.1nmol.l^{-1}; control vs 12.1 ± 5.7nmol.l^{-1}; occlusion, $p<0.01$). In contrast to adrenaline, plasma nor-adrenaline concentration was highest immediately after exercise, irrespective of condition. There was a 16-fold increase from rest to 22.8 ± 8.4nmol.l^{-1} in the control and a 10-fold increase from rest to 15.1 ± 7.9nmol.l^{-1} in the occlusion condition ($p<0.01$). Thereafter, the concentration of nor-adrenaline remained greater than at rest, irrespective of condition, in all samples taken including the 10min post-exercise sample ($p<0.01$) (Figure 6.9). Plasma nor-adrenaline concentration was found to be most highly correlated with blood pH in the post-exercise sample ($r=-0.827$, $p<0.01$, in control and $r=-0.843$, $p<0.01$ in occlusion conditions).

Plasma electrolytes

Plasma potassium concentration increased ($p<0.05$) after sprint 7 from 3.7 ± 0.4mmol.l^{-1} at rest to 4.4 ± 0.3mmol.l^{-1} in the control condition and from 3.9 ± 0.6mmol.l^{-1} to 4.4 ± 0.9mmol.l^{-1} in the occlusion condition and remained elevated in both conditions following completion of the exercise-test (Figure 6.10). During the subsequent recovery period, potassium concentration fell below resting values reaching its lowest value ($p<0.01$) 10min after exercise (3.3 ± 0.3mmol.l^{-1} in control and 3.4 ± 0.5mmol.l^{-1} in occlusion). Plasma sodium concentrations in response to exercise were not different from resting, irrespective of condition (Figure 6.11).

Change in plasma volume

The first seven sprints resulted in similar decreases in plasma volume, irrespective of condition, and were 10.1 ± 4.3% in control and 9.5% ± 4.5% in the occlusion condition (n.s.). The percentage decrease in plasma volume reached its greatest value immediately after exercise in the control condition (13.1 ± 4.4%) and at 3min post-exercise in the occlusion condition (14.1 ± 5.3%). These changes were not different between conditions.

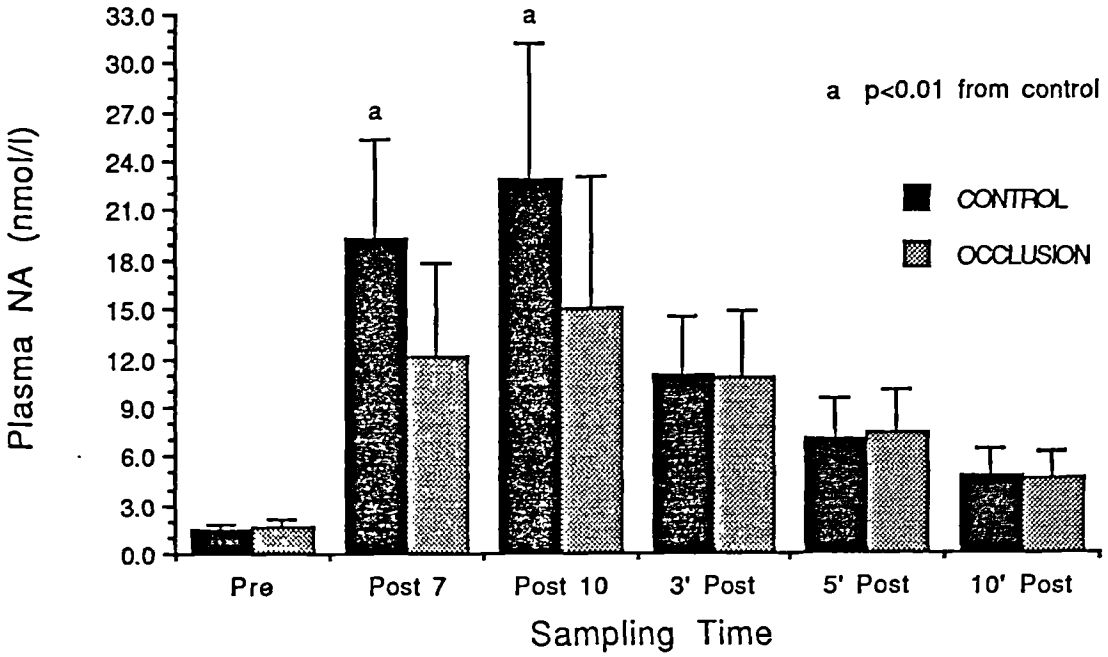


Figure 6.9. Plasma nor-adrenaline concentrations (nmol/l) during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

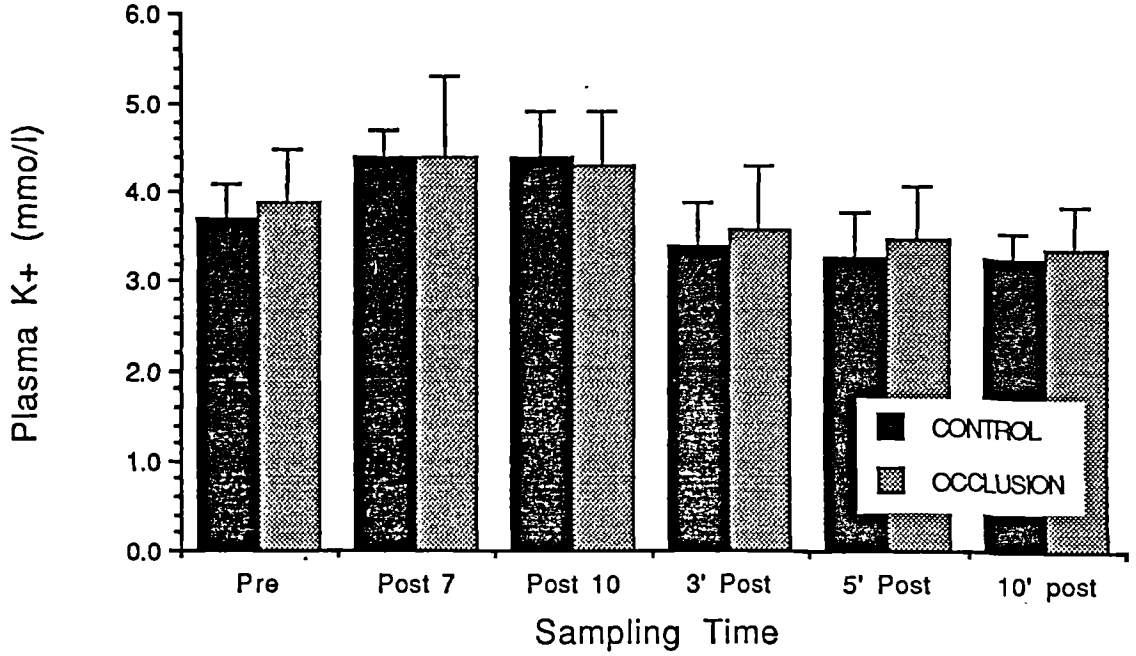


Figure 6.10. Plasma potassium concentrations (mmol/l) during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

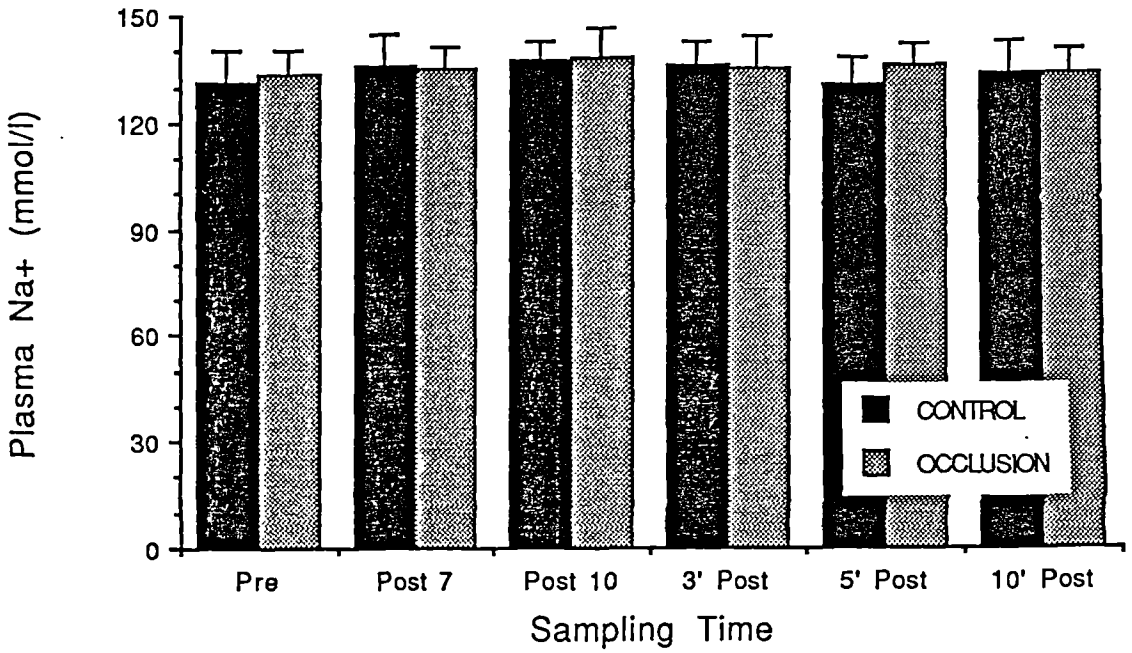


Figure 6.11. Plasma sodium concentrations (mmol/l) during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

Respiratory variables

The variations in ventilation (VE) during each 30s recovery period and during the 15min post-exercise recovery period after the exercise-test are shown in Figure 6.12 for both experimental conditions. The volume of expired air increased sharply over the first four sprints from 8.8 $\pm$ 1.8 $l.min^{-1}$ at rest to 85.2 $\pm$ 18.4 $l.min^{-1}$ in the control condition and from 9.0 $\pm$ 1.2 $l.min^{-1}$ to 73.6 $\pm$ 19.8 $l.min^{-1}$ in the occlusion condition (n.s.). Thereafter the changes in VE as the test progressed were small. In the control condition ventilation was higher after the eighth and ninth sprints ($p<0.01$) compared with occlusion, but all other expired air samples showed no differences between conditions. Following completion of the exercise test VE decreased rapidly but remained above resting values ($p<0.01$) throughout the 15min recovery period (14.3 $\pm$ 2.6 $l.min^{-1}$ in control and 13.9 $\pm$ 4.2 $l.min^{-1}$ in occlusion, n.s.).

As with ventilation, oxygen uptake (VO_2) increased sharply over the first four sprints from 0.3 $\pm$ 0.1 $l.min^{-1}$ at rest to 2.5 $\pm$ 0.5 $l.min^{-1}$ in the control and from 0.3 $\pm$ 0.1 $l.min^{-1}$ to 1.8 $\pm$ 0.5 $l.min^{-1}$ in the occlusion condition ($p<0.01$). Thereafter there were small variations in oxygen uptake until completion of the exercise-test but VO_2 was always higher in the control compared to the occlusion condition ($p<0.01$) (Figure 6.13). This difference is also reflected in the total volume of oxygen consumed over the recovery periods between sprints which was in absolute values 12.2 $\pm$ 2.5 l in the control and 9.1 $\pm$ 2.2 l in the occlusion condition ($p<0.01$). At the end of exercise the VO_2 fell rapidly during the first five minutes of recovery but was still higher than resting values ($p<0.01$) at 15min post-exercise (0.4 $\pm$ 0.1 $l.min^{-1}$ in control and 0.4 $\pm$ 0.1 $l.min^{-1}$ in occlusion). The total oxygen consumed in the 15min post-exercise recovery period was 9.0 $\pm$ 1.4 l in the control and 8.3 $\pm$ 2.2 l in the occlusion condition (n.s.). Both VE and VO_2 correlated ($p<0.01$) with PPO, MPO and average MPO, in both experimental conditions.

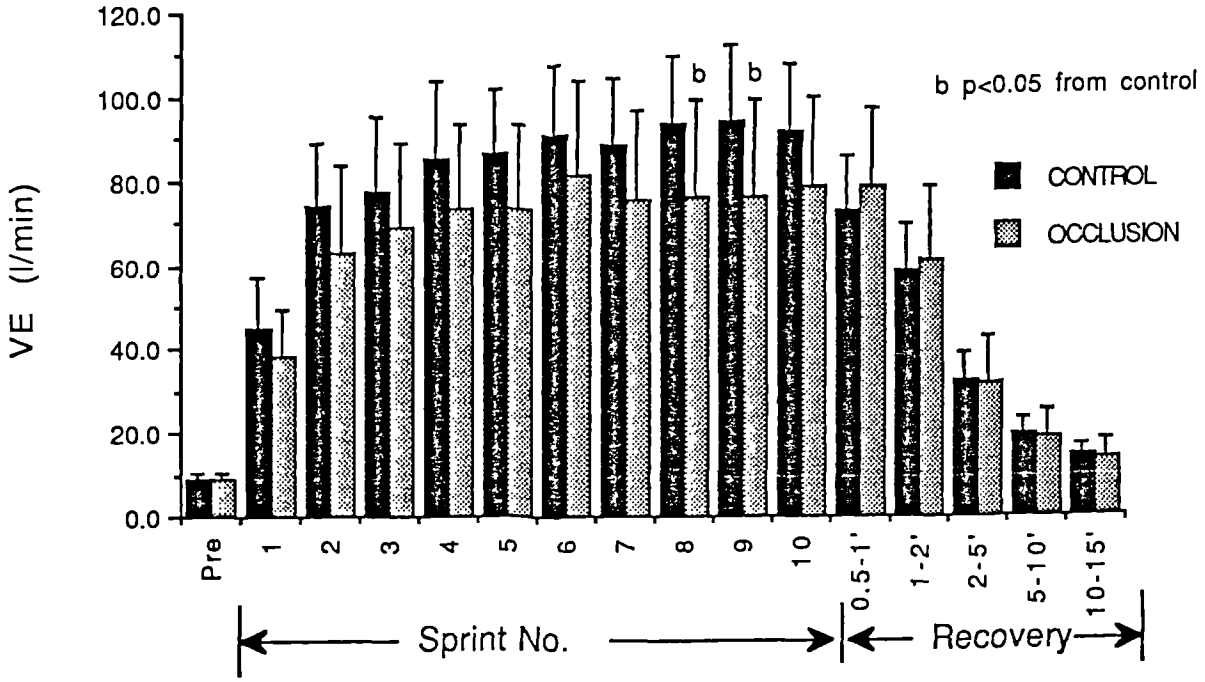


Figure 6.12. Ventilation (l/min) during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

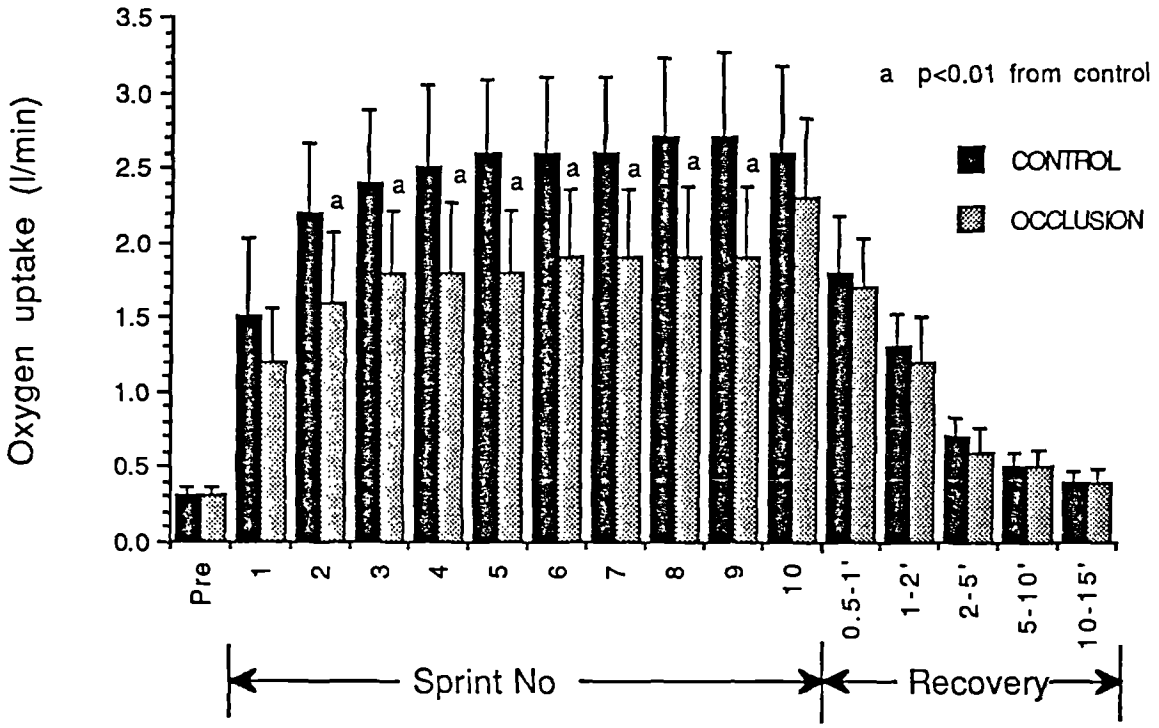


Figure 6.13. Oxygen uptake (l/min) during exercise and recovery in the control and occlusion conditions (mean ± s.d., n=13).

6.4 DISCUSSION

The changes in power outputs generated by the subjects in the control condition over the ten 6s sprints are similar to those observed in study 1 (Chapter 4) and demonstrate that considerable decrements in performance occur during this type of maximal intermittent exercise. However, these changes in performance were not as pronounced as those observed when the blood circulation to the legs was occluded during the recovery intervals between sprints.

Several possibilities exist to explain the greater fatigue seen in the occlusion compared with the control condition. It was suggested previously (study 1, Chapter 4) that despite a marked reduction in the PCr concentration after the first 6s sprint, the contribution of PCr degradation to anaerobic ATP resynthesis during this type of intermittent maximal exercise would be considerable, even after ten maximal 6s sprints. This would imply that, with intact circulation to the legs, a significant rate of PCr resynthesis would occur during each recovery interval despite its short duration (30s). In contrast, in the present study, by arresting the circulation to the thighs, the leg muscles could be considered as a closed system during each recovery interval compared with the control condition. A reduction therefore, in the availability of oxygen to the working muscle could be expected in that condition. The significantly lower values in VO_2 obtained in the occlusion condition, during the recovery intervals over the entire test, support this idea. As the rate of PCr resynthesis within the muscle cell is thought to be regulated primarily by the availability of ATP from oxidative metabolism, at least during the early phase of recovery (Harris et al, 1976; Sahlin et al, 1979; Hultman et al, 1981; Ren et al, 1988), it would appear that PCr resynthesis was inhibited during the recovery intervals as a result of arrested blood flow to the leg muscles. Consequently, the contribution from PCr degradation to the total energy production after the first sprint was reduced as the test was progressed in the occlusion condition compared with the control. If this was the case then muscle glycogen must have been the main energy source for muscle contraction during the subsequent sprints in the occlusion experiment.

A decrease in PCr content to the extent found in study 1 (Chapter 4) over the first 6s sprint would be expected to result in a significant elevation of intramuscular Pi concentration. Thereafter, as the circulation to the legs was occluded, thus inhibiting resynthesis of PCr, it would appear that the amount of Pi available for activation of phosphorylase in the a form would possibly be more than sufficient compared with the control condition. It could be expected therefore, that in the subsequent exercise bout the contribution of glycogen degradation to total ATP resynthesis should be greater under ischaemia than with intact circulation. Evidence in support of this suggestion is obtained from a recent study which showed that occlusion of the circulation to the legs for 60s between two bouts of electrical stimulation (20Hz) resulted in a 30% increase in the glycogenolytic rate in the second contraction compared with the first (Ren et al, 1988). In that study, when the second bout of exercise was preceded by 60s of intact circulation, the glycogenolytic rate remained unchanged. This possibility may explain partly why the decline in power output in sprint 2 following circulatory occlusion was not so marked from sprint 1 as was the case in the subsequent exercise bouts. Of course, it is also possible that the first sprint did not result in complete depletion of PCr as previously shown (study 1, Chapter 4). Thus, there may have been sufficient PCr available to make a significant contribution to ATP production in the second sprint.

Despite the possibility of an initial enhanced glycogenolytic rate in sprint 2 in the occlusion condition, it could be argued that as the test continued the rate of glycogen degradation to anaerobic ATP resynthesis was lower compared with the control condition. This could be explained as the combined effect of two factors. Firstly, it is likely that during ischaemia a reduction of available Pi necessary for activation of phosphorylase occurred. Indeed, accumulation of hexose monophosphates would remove a fraction of the Pi liberated as a result of PCr degradation during the initial exercise bouts of the test. Although some resynthesis of PCr is expected to occur during inflation and deflation of the cuffs, it seems unlikely that the amount resynthesised would be sufficient to provide sufficient Pi for glycogen degradation. In contrast with intact circulation experiments, the decrease in the

concentration of organic phosphates during each recovery interval is linked to resynthesis of PCr, the degradation of which in the subsequent exercise bout will liberate Pi.

It is also possible that during ischaemia, conversion of phosphorylase a to b form occurs in skeletal muscle, as previously suggested (Chasiotis and Hultman, 1983). Adrenaline is a potent stimulus for transformation of phosphorylase b to a form (Newsholme and Start, 1973). The elevated concentrations of plasma adrenaline observed during the exercise-test in the occlusion condition, would suggest that a greater fraction of phosphorylase would be in the a form compared with the control condition. It could be argued however, that the higher adrenaline concentration under ischaemia may reflect a reduced distribution as a result of circulatory occlusion to the legs and not an increased release from the adrenal medulla. Further support of this suggestion is provided from the findings in study 2 (Chapter 5) which showed an inverse relationship between blood pH and catecholamine response to exercise. If this is also the case in the present study then it would be expected that in the control experiment, adrenaline concentrations should be higher during exercise than during the ischaemia experiment as a result of the lower blood pH that was noted in the former condition. This however was not seen in the present study possibly suggesting an increased adrenaline uptake by skeletal muscle with intact circulation. Of course the possibility that the higher adrenaline concentration in the occlusion condition may be a reflection of greater stress experienced by the subjects cannot be dismissed.

The effectiveness of the occlusion of blood supply to the legs is also reflected by the considerably lower nor-adrenaline concentrations that were observed in comparison with the control condition. Indeed, it has been suggested previously (Christensen and Galbo, 1983) that the increases in nor-adrenaline concentration observed during exercise may originate from sympathetic nerve endings in skeletal muscle. Evidence in support of this suggestion derived from a recent study (Savard, Strange, Kiens, Richter, Christensen and Saltin, 1987) which showed that nor-adrenaline spillover (i.e. the rate at which the nor-adrenaline released from nerve endings enters the circulation) during one-leg

exercise, at 50% and 100% of maximum work load, was larger in the exercising leg compared with the resting leg. This result is supported in the present study in that nor-adrenaline concentration was lower in the occlusion condition. This is a consequence of nor-adrenaline not being able to escape from the occluded muscle.

By arresting the circulation to the legs during the recovery intervals it would appear that a reduced transport of metabolites such as H^+ produced in muscle, would occur. Thus, the possibility exists that the lower glycogenolytic rate in the occlusion condition compared with control was due to a greater acidotic environment in the former condition. There was a significantly smaller fall in blood pH and increase in blood lactate in the occlusion condition compared with control over the exercise-test, suggesting a reduced efflux of H^+ and lactate in the former condition. However, if the metabolic environment was more acidic under ischaemia, then one would expect to see a greater change in the acid-base balance of the blood during the recovery period following completion of the final sprint when the circulation to the legs was restored. This was not seen in the present study. In fact there was a trend of greater lactate concentrations in the control condition post-exercise in comparison with the occlusion (Figure 6.6) despite the possibility of a greater lactate oxidation within the active muscle that would have occurred as a result of intact circulation.

It is of interest to note however, that despite occlusion of the circulation to the legs during each recovery interval (30s) considerable efflux of H^+ and lactate occurred during the overall exercise period, which was 60s duration in total. This observation indicates that the rates of H^+ and lactate efflux during sprinting are very high. It further supports the findings of study 2 (Chapter 5) in which the circumstantial evidence suggests that blood alkalosis could not improve sufficiently the concentration gradient of H^+ from muscle to blood to increase efflux.

That the decline in power output seen in the present study in the occlusion condition could be attributed primarily to a dramatic reduction in the rate of ATP production is further supported in the

third experimental condition in which restoration of the circulation after sprint 7, resulted in considerable improvement in power output over the subsequent exercise bouts. The metabolic events during the exercise-test would appear to be as follows. In the first bout of maximal exercise ATP is derived from the breakdown of PCr and anaerobic glycogenolysis (study 1, Chapter 4). As a result of circulatory occlusion to the legs during each recovery interval the availability of oxygen to the muscle is reduced and PCr resynthesis is inhibited (Ren et al, 1988). Consequently, glycogenolysis is the only available means of producing ATP. However, the formation of hexose monophosphates remove a proportion of Pi, which has been implicated as a potent activator of phosphorylase, thus reducing glycogen degradation possibly to very low rates. Under the prevailing conditions total ATP turnover is reduced. Restoring the blood flow to the legs during recovery, PCr is resynthesised and contributes as an energy source in the subsequent exercise bout. In addition, liberation of Pi as a result of PCr degradation reactivates glycogenolysis, and power output recovers to values almost equal to those attained in the control condition. If these processes did occur in the muscle under ischaemia then the importance of PCr in this condition, as a possible metabolic regulator, is evident. Moreover, the rapid recovery in power output that was seen in sprint 8, by restoring the circulation in the preceding recovery interval (30s), would further support previous suggestions (study 1, Chapter 4 and Harris et al, 1976) that resynthesis of PCr with intact circulation is a very rapid process during the early phase of recovery which is dependent on oxygen availability.

It would also appear that the fatigue process in the occlusion condition may not be associated with an H^+ mediated inhibition of the ATP generating process. Following restoration of the circulation after sprint 7, a decline in H^+ concentration in muscle would be expected as a result of its enhanced efflux to the blood. However, it is possible that this decline in H^+ is balanced by a release of H^+ due to PCr resynthesis. Indeed, the high rates of PCr resynthesis during the early phase of recovery which have been observed in some studies, (Harris et al, 1976) and suggested in others (study 1, Chapter 4) at a relatively low muscle pH (study 2, Chapter 5) would indicate that considerable

amount of H^+ is released as a result of PCr resynthesis. It could be expected therefore, that restoring the blood flow after sprint 7, pH would remain that low during the subsequent 30s of recovery. However, as power output had recovered to high values over sprint 8, it would appear that it was not limited by a low intramuscular pH. Indeed, the data of Sahlin and Ren (1989) have shown that despite a persistently low muscle pH (calculated), contraction force was completely restored after 2min of recovery with intact circulation.

It was suggested in study 1 (Chapter 4) in which an identical exercise protocol was used, that the decline in power output during this type of exercise with intact circulation to the legs was accompanied by decreased contribution of anaerobic glycogenolysis to ATP resynthesis. It was argued in the present study that, under ischaemia during each recovery interval, this was possibly the result of a reduced availability of Pi for phosphorylase activity rather than an H^+ mediated mechanism. Whether a similar mechanism could explain the reduction in the glycogenolytic rate in the control condition is not known. Nevertheless, it was found in study 1 (Chapter 4) that muscle lactate concentrations were high over the ten 6s sprints. It was also estimated, from the muscle lactate concentrations, that pH would probably be about 6.6 although it was found to be 6.8 at comparable lactate levels in study 2 (Chapter 5). It would appear therefore, that the recovery intervals during the exercise-test in the control condition would be characterised by a high lactate content and a rapid resynthesis of PCr. Degradation of PCr, with concomitant liberation of Pi, would rephosphorylate ADP at a high rate and would restore the ATP generating processes despite a relatively low intramuscular pH. Under the prevailing conditions the reduction in the glycogenolytic rate in the control condition could be explained as a result of increased concentration of $H_2PO_4^-$ affecting either phosphorylase activity (Chasiotis, 1983) or release of Ca^{2+} from the sarcoplasmic reticulum (Wilson et al, 1988).

In the present study, no differences were found in the concentration of plasma electrolytes between experimental conditions. Thus, the greater fatigue observed in the occlusion condition compared with control would

not seem to be associated with greater falls in membrane potential, reduced excitability or changes in membrane transport mechanisms affecting the distribution of electrolytes across the cell membrane.

However, the possibility exists that the larger fall in power output, seen in the occlusion condition, resulted from a greater depletion of the muscle ATP content. It has been suggested that when the capacity to rephosphorylate ADP is reduced, then ADP and AMP will increase, thereby activating AMP deaminase (Katz et al, 1986; Sahlin, 1986). The action of AMP deaminase is thought to be beneficial to the cell by reducing the fall in the ATP to ADP ratio. As deamination of AMP to IMP is irreversible, its potential for immediate resynthesis back to ATP is lost. Consequently, progressive decrements in the muscle ATP store would occur in the occlusion condition as a result of the need for a high ATP turnover coupled with a low PCr content. This would eventually limit the muscle's ability to accelerate the rate of energy demand, thus resulting in greater fatigue. However, if ATP depletion was the primary reason for the greater fatigue seen in the occlusion condition then, restoration of the circulation following completion of sprint 7 would not have resulted in such a dramatic recovery in power output. Evidence in support of this suggestion is provided from a study (Hultman et al, 1981) in which following 75s of electrical stimulation (20Hz) and arrested blood flow, the circulation to the legs was restored by deflating and inflating the tourniquet. It was found that PCr was resynthesised but the ATP content was unchanged and remained at a reduced level. Possibly, all the ATP resynthesis was used for PCr resynthesis.

It is generally accepted that during short-term high-intensity exercise increases in plasma ammonia reflect deamination of AMP to IMP and ammonia in contracting muscle and net diffusion of the latter in the blood. In the present study no differences in peak plasma ammonia concentration were found between the occlusion and control conditions, which occurred at 5min post-exercise, irrespective of condition. However, whether this finding reflects a similar reduction in TAN loss in both conditions, in view of the fact that the pH gradient between tissues determines their relative ammonia concentrations and the

direction of ammonia movement between intra- and extracellular compartments (Mutch and Banister, 1983; Harris and Dudley, 1989), it can be argued. Nevertheless, the high plasma ammonia concentrations found in the present study do suggest that considerable reduction in TAN must have occurred during this type of exercise, irrespective of condition.

The findings of the present study provide some evidence in support of the hypothesis that the primary cause of fatigue is due to insufficient ATP formation for the demand during this type of exercise. It was shown that when energy cannot be exchanged at high rates required to maintain a high ATP turnover, as was suggested for the case in the occlusion condition, the decline in power output was greater compared with the control condition. Moreover, in further support of this proposal, by restoring the circulation to the legs after sprint 7 there was a rapid recovery in power output to values similar to control.

7. GENERAL SUMMARY

The purpose of the studies undertaken in this thesis, was to describe the metabolic changes associated with intermittent maximal exercise in order to examine the relationship between muscle metabolism and fatigue. The exercise protocols consisted of repeated exercise periods of brief duration (i.e. 6-10s) interrupted by 30s recovery intervals. Fatigue was defined as the decline in power output during successive exercise periods.

It was shown in Study 1 that the energy required to support the high power output generated by the subjects over the first bout of exercise (6s), was primarily provided by almost equal contribution from PCr degradation and anaerobic glycogenolysis. However, in the tenth bout of exercise (6s) there was no change in muscle lactate concentration although the average power output was 73% of that generated in the first sprint. In face of a considerable reduction in the contribution of anaerobic glycogenolysis to ATP resynthesis, it was suggested that during the last bout of exercise, power output was supported by energy which was derived from PCr degradation and an increased aerobic metabolism. However, whether the reduced rate of ATP resynthesis from anaerobic glycogenolysis was the primary cause of fatigue or was secondary to inhibited ATP utilisation is not known.

Muscle acidosis as a result of lactic acid accumulation has been implicated as the cause of fatigue due to either direct or indirect effects on the contractile mechanism or through indirect involvement on the ATP generating processes (see Section 2.7 in Chapter 2). The increase in muscle lactate concentration found at the end of the exercise-test in Study 1, has been shown to be associated with large decreases in muscle pH (Hultman and Sahlin, 1980). Indeed, it was estimated from muscle lactate concentrations that the muscle pH at the end of the last sprint was 6.59. Therefore, it would appear that acidosis resulting from maximal anaerobic glycogen degradation, particularly at the early stages of the multiple-sprint test, could explain the decline in power output.

This possibility was examined in Study 2 by ingesting solution of sodium bicarbonate prior to exercise. In doing so, it was thought that it may be possible to delay the onset of fatigue by increasing the rate of H^+ efflux from muscle to blood, particularly during the recovery intervals, as a result of an increased buffering capacity of the blood. Although, oral ingestion of sodium bicarbonate resulted in significant shifts in acid-base balance of the blood prior to exercise and a greater efflux of H^+ during the exercise test compared with NaCl ingestion, it had no effect on muscle metabolism. Moreover, the considerable decrements in power output that occurred during this type of intermittent maximal exercise (45%) were not associated with the low muscle pH values that have been recorded in other studies during exercise of lower intensity to exhaustion (see Section 2.4, in Chapter 2). In this study muscle pH, as determined by the homogenate technique, was measured to be 6.8, irrespective of experimental condition, at comparable lactate concentrations to those found in Study 1. These findings may suggest that during maximal exercise, a relatively small decrease in muscle pH is associated with a large reduction in the generation of power output. Whether this decline in power output was the result of the presence of increased H^+ on the contractile process per se or on the energy provision by glycolysis or both, is at present unknown.

Nevertheless, a common finding of the first two studies was that the contribution of PCr to anaerobic ATP resynthesis, during intermittent maximal exercise, was significant. Therefore, the important role of PCr in energy production during this type of exercise was indirectly examined by occluding the circulation of the legs during each recovery interval, thereby inhibiting resynthesis of PCr. It was shown that when energy cannot be generated at high rates, as was suggested in the occlusion condition, the decline in power output is greater compared with the control condition. It was suggested therefore, that the cause of fatigue is due to insufficient ATP formation for the demand during this type of exercise. In further support of this proposal is the observation that when the circulation to the legs was restored for 30s, there was a rapid recovery in power output to values similar to control. These results point to PCr as a key regulator of ATP resynthesis during intermittent exercise of maximal intensity.

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APPENDIX A

Fluorimetric method for the determination of lactate in blood

Principle:

$$\text{Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

Reagents:

Buffer: Hydrazine 1.1mol.l^{-1}, EDTA 1mmol.l^{-1}, pH 9.0

Cofactor: NAD (Grade I)

Enzyme: LDH 5500 U.ml^{-1} (undiluted)

Standard: Lactic acid 1mol.l^{-1} diluted to 20mmol.l^{-1} stock

Additional Reagent: 0.07 mol.l^{-1} HCl

Standards:

Concentration, mmol.l^{-1}	0	2	5	10	15	20
µl lactic acid 20mmol.l^{-1}	0	200	500	1000	1500	2000
µl 2.5% $HClO_4$	2000	1800	1500	1000	500	0

Reaction Mixture:

Buffer	1ml
NAD	2mg
LDH	10µl

Procedure:

1. Add 200µl reaction mixture to 20µl aliquots of samples, standards and perchloric acid blanks.
2. Incubate for 30mins at room temperature.
3. Add 1ml 0.07mol.l^{-1} HCl and read fluorescence.

Determination of plasma catecholamine concentrations by HPLC with electrochemical detection

Principle: Simple liquid-solid extraction of the catecholamines onto alumina, followed by their elution with dilute acid.

Reagents:

Mobile Phase: 37.8g monochloraecetic acid, 11.4g NaOH, 3.0g EDTA, 0.36g Na^+- octanesulphonic acid, 4 litres HPLC H_2O, pH 3.0 (degased every 4 days)

Phosphate Buffer: $0.1 mol.l^{-1}$, pH = 7.0

Tris Buffer/ EDTA: $1.5 mol.l^{-1}$, pH = 8.6

Additional Reagents: $0.1 mol.l^{-1}$ $HClO_4$, acid washed aluminium oxide

Standards: Noradrenaline bitartrate - $1.0 mmol.l^{-1}$ stock (NA)
Adrenaline bitartrate - $0.4 mmol.l^{-1}$ stock (AD)
3,4-dihydroxybenzylamine - $1.0 mmol.l^{-1}$ stock (IS)

Stock standards were diluted as follows:

Stock solution	100µl	$10 nmol.ml^{-1}$ NA
+		$4 nmol.ml^{-1}$ AD
$0.1 mol.l^{-1}$ $HClO_4$	9.9ml	$10 nmol.ml^{-1}$ IS

Working standards were prepared daily from the diluted standards (kept at 4^oC), as follows:

20µl of diluted standard + 1.98ml of $0.1 mol.l^{-1}$ $HClO_4$ ---> $100 pmol.ml^{-1}$ NA, $40 pmol.ml^{-1}$ AD, $100 pmol.ml^{-1}$ IS (reaction mixture)

Procedure:

1. Thaw plasma sample. Can be speeded up by using water bath.
2. Spin sample for 5min at 5000rpm.
3. Place 50mg alumina in each glass tube (washed in clean nitric acid).
4. Place 1ml Trisbuffer/EDTA in each tube.
5. Add 3ml of plasma.
6. Add 200µl of 100pmol.ml^{-1} IS (i.e. 20pmol in 200ul) for post-exercise samples and 100µl of 100pmol.ml^{-1} IS (i.e. 10pmol in 100ul) for resting samples.
7. Spin in whirlimix and then shake for 10min.
8. Remove plasma.
9. Wash alumina twice with double distilled H_2O.
10. Transfer alumina to 'catecholamine tubes', loaded with RC58 membranes and a receiver tube.
11. Remove excess H_2O from 'catecholamine tubes'.
12. Spin to dryness and remove H_2O from the receiver tube.
13. Add 100µl of 0.1mol.l^{-1} $HClO_4$ on alumina to elute catecholamines.
14. Mix in whirlimix.
15. Spin for 2min at 5000rpm.
16. Inject 100µl of the acidic extract in the receiver tube for resting samples and 2 x 50µl for post-exercise samples.

To calculate the recovery of catecholamines from alumina (accepted range >50-60%) the above procedure was performed using 2ml phosphate buffer + 200µl of reaction mixture prior to plasma analysis (expected amounts in 50µl of acid extract injected; NA = 10pmol, AD = 4pmol, IS = 10pmol). The raw peak height responses (Physics, Integrator SP4290) were compared with those obtained by injecting 50µl of reaction mixture directly, without prior extraction (expected amounts in 50µl of reaction mixture; NA = 5pmol, AD = 2pmol, IS = 5pmol). In addition the peak height responses per pmol (RMR) of IS/NA and IS/AD were determined and used to calculate the concentration of catecholamines in the plasma samples as follows:

$$\text{concentration (x)} = \left[\frac{\text{response x}}{\text{response IS}} \times \text{amount IS X RMR}\right]/\text{Vol of sample}$$

APPENDIX B

Methods for the determination of soluble and insoluble glycogen in muscle

Acid-insoluble glycogen

1. Add 100µl of HCl 1mol.l^{-1} for each mg of muscle powder to the muscle pellet left in the bottom of the tube after the extraction procedure.
2. Mix and heat in boiling water bath for 2 hours.
3. Spin and cool to room temperature.
4. Determine concentration of glucosyl units photometrically by GOD-period method i.e. incubate 10µl aliquots of blank (D.W.), standard (0.505mmol.l^{-1} Boehringer) and supernatant for 30 minutes.
5. Read on spectrophotometer at 436nm.

NB May need 10µl aliquots in 2ml GOD.

Acid-soluble glycogen

1. Add 100µl HCl 1mol.l^{-1} to 20µl aliquots muscle extract.
2. Mix and heat in boiling water bath for 2 hours.
3. Spin cool and neutralise with 15µl of NaOH 6mol.l^{-1}.
4. Determine concentration of glucosyl units on the hydrolyzed extract by G6P-DH/HK fluorimetric method for glucose (glucose and G6P must be subtracted from the results).

Fluorimetric method for the determination of ATP and PCr in muscle

Principle:

$$ATP \xrightarrow{HK} ADP \qquad NADP^+ \xrightarrow{G6P\text{-}DH} NADPH + H^+$$

$$Glucose \xrightarrow{HK} Glucose\text{-}6\text{-}phosphate \xrightarrow{G6P\text{-}DH} 6\text{-}phosphoglucono\text{-}Lactone$$

$$ADP + PCr \xrightarrow{CK} ATP + Cr$$

Buffer:	Tris-HCl 50mmol.l^{-1}, pH 8.1 with 0.02% BSA
Cofactor:	NADP 5mmol.l^{-1}
Enzymes:	G6P-DH 14U.ml^{-1}; HK 28 U.ml^{-1}; CK 910 U.ml^{-1}
Reagents:	ADP 10mmol.l^{-1}; Glucose 10mmol.l^{-1}
	Dithiothreitol 50mmol.l^{-1}
	$MgCl_2$ 100mmol.l^{-1}
Standard:	ATP 2mmol.l^{-1}
Additional Reagents:	Tris-HCL buffer pH 8.1% with 0.02% BSA
	Carbonate buffer 20mmol.l^{-1}, pH 10.0

Standards:						
Concentration, µmol.l^{-1}	0	25	50	100	150
µl 2mmol.l^{-1} ATP	0	25	50	100	150
µl D.W.	2000	1975	1950	1900	1850

Reaction Mixture:				
Buffer	1ml	Dithiothreitol	10µl
NADP	10µl	$MgCl_2$	50µl
ADP	10µl	G6P-DH	5µl
Glucose	10µl		

Procedure:

1. Add 100µl D.W. to 17µl extract and pipette 10µl aliquots into 3 pairs of tubes.
2. Add 200µl reaction mixture to one pair of tubes and D.W. blanks (G6P).
3. Add 5µl HK per ml remaining buffer and add 200µl reaction mixture to second pair of tubes, standards and D.W. blanks (G6P + ATP).
4. Add 10µl CK per ml remaining buffer and add200µl reaction mixture to third pair of tubes and D.W. blanks (G6P + ATP + PCr).

5. Incubate for 20 minutes at room temperature, add 1ml carbonate buffer and read fluorescence.
6. Calculate ATP and PCr by subtraction. No attempt is made to determine G6P.

Fluorimetric method for the determination of ADP and AMP in muscle

Principle:

$$NADH+H^+ \text{------} > NAD^+$$

$$\text{Pyruvate} \xrightarrow[\text{LDH}]{\text{----------}} \text{Lactate}$$

$$ADP \text{ -->} ADP$$

$$\text{Phosphoenol pyruvate} \xrightarrow[\text{PK}]{\text{----------}} \text{Pyruvate}$$

$$AMP + ATP \xrightarrow[\text{MK}]{\text{--------}} 2\ ADP$$

Buffer:	Low fluorescence imidazole 50mmol.l^{-1} pH 7.0					
Cofactor:	NADH 2mmol.l^{-1} (grade I)					
Enzymes:	MK 36 U.ml^{-1}; PK 75 U.ml^{-1}; LDH 5500 U.ml^{-1}					
Reagents:	ATP 2mmol.l^{-1}; PEP 2mmol.l^{-1}; $MgCl_2$ 100mmol.l^{-1}					
	KCl 3mol.l^{-1}; EDTA 100 mmol.l^{-1}					
Standard:	ADP 2mmol.l^{-1}					
Additional						
Reagents:	Carbonate buffer 20mmol.l^{-1}, pH 10.00					
	Tris-HCl buffer 20mmol.l^{-1}, pH 8.1 with 0.02% BSA					
Standards:	Concentration, µmol.l^{-1}	0	10	30	50	70
	µl ADP 2mmol.l^{-1}	0	10	30	50	70
	µl D.W.	2000	1990	1970	1950	1930

Reaction Mixture:	Buffer	1ml	NADH	3µl
	$MgCl_2$	20µl	PEP	5µl
	KCl	25µl	EDTA	2µl
	ATP	2.5µl		

Procedure:

1. Dilute extract (17µl extract + 100µl D.W.), mix.
2. Pipette 10µl aliquots of extract into 3 pairs of fluorimeter tubes.
3. Add 0.5µl LDH per ml reaction mixture remaining and add 200µl to one set of tubes and D.W. blanks (extract blanks + pyruvate).
4. Add 10µl PK per ml buffer remaining add 200µl to one set of tubes and standards (extract blanks + pyruvate + ADP).
5. Add 40µl MK per ml remaining buffer and add 200µl to remaining set of tubes (extract blanks + pyruvate + ADP + 2 AMP).
6. Incubate for 20 minutes at room temperature and read fluorescence.
7. Calculate pyruvate, ADP and 2 AMP by subtraction.

Fluorimetric method for the determination of creatine in muscle

Principle:

$$\text{Creatine} + \text{ATP} \xrightarrow{\text{CK}} \text{PCr} + \text{ADP}$$

$$\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{ATP} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

Buffer: Low fluorescence imidazole 50mmol.l^{-1} pH 8.5

Cofactor: NADH 2mmol.l^{-1} (grade I)
PEP 2mmol.l^{-1}

Enzymes: LDH 40 U.ml^{-1}; PK 75 U.$ml.^{-1}$
CK 3600 U.ml^{-1}

Reagents: ATP 10mmol.l^{-1}; $MgCl_2$ 100mmol.l^{-1}
KCl 3mol.l^{-1}

Standard: 2 mmol.l^{-1} creatine

Additional Reagents: Carbonate buffer 20mmol.l^{-1}, pH 10.0,
EDTA 100 mmol.l^{-1}

Standards:

Concentration, µmol.l^{-1}	0	50	100	150
µl 2mmol.l^{-1} creatine	0	50	100	150
µl D.W.	2000	1950	1900	1850

Reaction Mixture:

Buffer	1ml	NADH	15µl
$MgCl_2$	50µl	EDTA	1µl
KCl	10µl	LDH	5µl
ATP	20µl	PK	10µl
PEP	25µl	CK	10µl

procedure:

1. Dilute extract (17µl extract + 100µl D.W.), mix.
2. To 10µl of standard and samples, add 200µl reaction mixture, mix.
3. Incubate for 30-40 minutes at room temperature; add 1ml carbonate buffer and read fluorescence.
4. Determine creatine by subtracting ADP and pyruvate from results.

Fluorimetric method for the determination of glucose, glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate in muscle

Principle:

$NADP^+ \rightarrow NADPH + H^+$

G6P ----------> 6-P-Guconolactone

ATP -->ADP

Glucose -------> G6P

HK

F6P ---------> G6P (PGI)

G1P ----------> G6P (PGluM)

Buffer: Tris-HCl 50mmol.l^{-1}, pH 8.1 with 0.02% BSA

Cofactor: NADP 5mmol.l^{-1}

Enzymes: G6P-DH 7 U.ml^{-1}; HK 28 U.ml^{-1}; PGluM 4 U.ml^{-1}
PGI 35 U.ml^{-1}

Reagents: Glucose 1, 6-diphosphate 250 umol.l^{-1}; $MgCl_2$ 100mmol.l^{-1}
Dithiothreitol 50mmol.l^{-1}; EDTA 100mmol.l^{-1}; ATP 10mmol.l^{-1}

Standard: G6P 2mmol.l^{-1}

Additional Reagents: Tris-HCl buffer 20mmol.l^{-1}, pH 8.1 with 0.02% BSA
Carbonate buffer 20mmol.l^{-1}, pH 10.0

Standards:	Concentration, µmol.l^{-1}	0	10	30	50	100	250
	µl G6P 2mmol.l^{-1}	0	10	30	50	100	250
	µl D.W.	2000	1990	1970	1950	1900	1750

(Standard range; resting samples 0-30, post exercise samples 30-250)

Reaction Mixture:				
	Buffer	1ml	EDTA	5µl
	NADP	10µl	G1,6-DP	2µl
	$MgCl_2$	10µl	G6-P-DH	10µl
	Dithiothreitol	10µl		

Procedure:

1. Pipette 10µl aliquots of samples into 4 sets of tubes.
2. Add 200µl of reaction mixture to standards and one set of tubes (G6P).
3. Divide remaining reaction mixture into 3 and add 5µl HK + 20µl ATP (glucose), 10µl PGluM (G1P) or 10µl GPI (F6P) per ml of buffer, respectively. Add 20µl of one of the 3 reaction mixtures to the 3 remaining sets of tubes and D.W. blanks.
4. Incubate for 30 minutes at room temperature, add 1ml carbonate buffer, read fluorescence and determine concentrations by subtraction.

Fluorimetric method for the determination of fructose 1,6-diphosphate and triose phosphate in muscle

Principle: Fructose 1,6-diphosphate ------> Dihydroxyacetone phosphate
aldolase

Dihydroxyacetone phosphate --> glyceraldehyde 3-phosphate
TPI

NAD^+ ---> NADH + H^+

Glyceraldehyde 3-phosphate -----> 1,3-Diphosphoglycerate
Glyceraldehyde 3P-DH

Buffer: Low fluorescence imidazole 50mmol.l^{-1}, pH 7.5
Cofactor: NAD 10mmol.l^{-1}
Enzymes: Aldolase 9 U.ml^{-1}; TPI 240 U.ml^{-1}; GAP-DH 360 U.ml^{-1}
Reagents: Sodium arsenate 100mmol.l^{-1}; EDTA 100mmol.l^{-1}
Mercaptoethanol 1mol.l^{-1} in 0.02 mol.l^{-1} KOH
Standard: Fructose 1,6-diphosphate 200umol.l^{-1}
Additional Reagents: Tris-HCl buffer 20mmol.l^{-1}, pH 8.1 with 0.02% BSA
Carbonate buffer 20mmol.l^{-1}, pH 10.0

Standards:	Concentration, µmol.l^{-1}	0	5	10	20	40
	µl F-1,6-diP 200umol.l^{-1}	0	50	100	200	400
	µl D.W.	2000	1950	1900	1800	1600

Reaction Mixture:	Buffer	1ml	Mercaptoethanol	2µl
	NAD	10µl	GAP-DH	4µl
	EDTA	10µl	TPI	10µl
	sodium arsenate	10µl		

Procedure:

1. Add 200µl reaction mixture to 20µl aliquots of samples and D.W. blanks (triose phosphates).
2. Add 3µl aldolase per ml remaining buffer and add 200µl of new reaction mixture to 20µl aliquots of samples, standards and D.W. blanks (F-1,6-diP + triose phosphates).
3. Incubate 50 minutes at room temperature.
4. Add 1ml carbonate buffer and read fluorescence.
5. Determine 2 F-1,6-diP by subtraction.

Fluorimetric method for the determination of pyruvate in muscle (by development of fluorescence in strong alkali)

Principle:

$$\text{Pyruvate} \xrightarrow[\text{LDH}]{\text{NADH+H}^+} \text{lactate}$$

Buffer: Imidazole buffer pH 7.0, 30mmol.l^{-1}

Cofactor: NADH 5mmol.l^{-1}

Enzymes: LDH 5500 U.ml^{-1}

Standard: Pyruvic acid 2mmol.l^{-1}

Additional Reagents: NaOH 6mol.l^{-1}, HCl 4mol.l^{-1}

Reaction Mixture:
1ml buffer
2ul NADH
2ul LDH

Standards:	Concentration, µmol.l^{-1}	0	10	30	50	100
	µl pyruvic acid 2mmol.l^{-1}	0	10	30	50	100
	µl D.W.	2000	1990	1970	1950	1900

Procedure:

1. Add 200µl reaction mixture without LDH to 10µl aliquots of samples and D.W. blanks (extract blank).
2. Add 200µl reaction mixture with LDH to samples, standards and D.W. blanks (pyruvate + extract blanks).
3. Incubate for 40 minutes at room temperature.
4. Add 20µl HCl 4mol.l^{-1} and leave standing for 5 minutes.
5. Add 1ml NaOH 6mol.l^{-1} and mix immediately.
6. Incubate for 60 minutes in the dark.
7. Read fluorescence and determine pyruvate by subtraction.

Fluorimetric method for the determination of lactate in muscle

Principle: $NAD^+ \rightarrow NADH + H^+$

Lactate ----------> pyruvate (LDH)

Buffer: Hydrazine 1.1mol.l^{-1}, pH 9.0 with 1mmol.l^{-1} EDTA

Cofactor: NAD (grade I)

Enzymes: LDH 5500 U.ml^{-1}

Standard: Lactic acid 1mol.l^{-1} diluted to 2mmol.l^{-1}

Additional Reagent: Carbonate buffer 20mmol.l^{-1}, pH 10.0

Standards:

Concentration, µmol.l^{-1}	0	50	100	150	750	1250
µl lactic acid 2mmol.l^{-1}	0	50	100	150	750	1250
µl D.W.	2000	1950	1900	1850	1250	750

(standard range: 0-150 for resting muscle; 0-1250 for post-exercise samples)

Reaction Mixture:

Buffer	1ml
NAD	2mg
LDH	10µl

Procedure:

1. Add 200µl reaction mixture to 10µl aliquots of samples, standards and D.W. blanks
2. Incubate for 30 minutes at room temperature.
3. Add 1ml carbonate buffer and read fluorescence.

APPENDIX C

Pilot Study: Changes in blood bicarbonate and standard bicarbonate concentrations, base excess and blood pH, 2.25h after the ingestion of $NaHCO_3$ (mean ± s.d, n=4).

VARIABLES	PRE-INGESTION	POST-INGESTION
HCO_3^- $mmol.l^{-1}$	26.4 ± 0.8	33.9 ± 4.5
Stand HCO_3^- $mmol.l^{-1}$	25.2 ± 0.9	30.1 ± 3.9
Base excess $mmol.l^{-1}$	1.2 ± 0.4	8.8 ± 3.9
pH	7.39 ± 0.03	7.46 ± 0.03