THE MAXIMAL LACTATE STEADY STATE DURING ENDURANCE EXERCISE IN HUMANS

By

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A Doctoral thesis submitted in partial fulfillment of the requirements for the award of Doctor of Philosophy of the University of Brighton

December 2009

To Mum, Dad, Katherine and Ashleigh – Thank you…..

‘Success is the ability to go from one failure to another with enthusiasm’ (Sir Winston Churchill)

ABSTRACT

The aim of this thesis was to understand the physiological mechanisms which regulate exercise at the Maximal Lactate Steady State (MLSS) and contribute to fatigue when exercise exceeds this intensity.

Chapter four demonstrated that the MLSS was sensitive to diurnal (time of day) variation. The lactate response at MLSS was significantly altered when comparing exercise in the evening (18:00) to morning (06:00) (4.88 ± 0.02 to 6.14 ± 0.07 mM respectively). This alteration was attributed to the circadian variation in body temperature which meant that lactate production was greatest when body temperature was at its highest.

Chapter five examined the intensity at MLSS as a training stimulus. Subjects either exercised continuously at MLSS or intermittently 0.5 $km \cdot h^{-1}$ above and below MLSS intensity. After eight weeks of training, both groups had significantly increased running speed at MLSS (~6%), the lactate threshold (~8%) and at $\dot{V}O_2max$ (~8%). The results of this study suggest that training at an intensity based on the MLSS is a powerful stimulus for improving aspects of sub-maximal and maximal exercise performance.

Chapter six demonstrated that exercise above the MLSS is characterized by a number of physiological mechanisms which may contribute to fatigue, in relation to, and independent from circulating lactate concentrations. Notably, pH, HCO_3^- and Bas Excess declined, whilst carbohydrate utilization and potassium concentrations

increased when exercise intensity increased above the MLSS. In addition, it appeared that there was a disproportionate increase in lactate compared to pyruvate concentrations. Taken together, the interpretation of the results from this chapter suggested that a number of mechanisms could potentially contribute to fatigue above the MLSS.

Chapter seven presented evidence that the principle mechanism for the increase in lactate concentrations above the MLSS appears to be due to saturation in lactate clearance capacity (Rd). This resulted in a disproportionate lactate rate of appearance (Ra) to Rd, and a net increase in lactate concentrations. In addition, glucose rate of appearance was elevated above the MLSS which helped to explain the elevated lactate (Ra) above the MLSS as provision of glucose for glycolysis increases.

Collectively this thesis identifies the MLSS as an important transition for submaximal endurance exercise and demonstrates that a number of physiological mechanisms involved in fatigue are increased when exercise progresses beyond this exercise intensity. The MLSS therefore has theoretical and practical function in exercise physiology as a model for exploring the mechanistic basis of muscular fatigue and the determinants of human exercise tolerance.

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ACKNOWLEDGMENTS

To my parents, Pat and Mike, sorry this has taken so long! There is no doubt that it would have failed a long time ago if it hadn't been for your love, support and understanding. I will always be grateful to you for everything you have done over the past twenty-nine years. Katherine, thank you for steering me away from a life of sport studies and recreation management, how wise you were. Thanks for always being interested and keeping me up-to-date with the goings on of Sir Frederic Osborn school.

To my darling Ashleigh, my best friend. You have had to put up with my struggles on a day-to-day basis. Hopefully I can return the favour one day. I love you dearly and will always be grateful to your colour organizer charts and writing blackmail approaches, you are a force to be reckoned with….

Thank you to my supervisors, Peter, Helen and Jamie for their support over the years. To Jamie for getting me started in the beginning, to Helen for keeping things on track and to Peter for opening a door to aspects of physiology that I never knew existed. For this I will always be grateful, even though you are a blue. Special thanks to Dr. Carl Hulson and Professor Asker Jeukendrup (University of Birmingham) for their help and advice with the IRMS samples, whilst Dan Wilkinson and Dr. Kenny Smith (University of Nottingham) are acknowledged for help and assistance with the GCMS samples

To Ols, Adam, Porky, Kirsten, Bush, Timmay, Cleeve, Craigos Nealos, Norbert and Neil M, thanks for all the great laughs and for leaving me with some happy lasting memories of the sunshine coast despite the recent darker days ☺

Thank you to Anne, Patrick, Ian, Dave, Alan and Ron for all your technical assistance, constant patience and for making the labs a fun place to work. I would also like to voice a special thank you to all of the subjects who volunteered their time and effort to give me the data for this thesis. I'm sorry for my cooking, sorry for my shouting, sorry that the university cannot pay recruits and sorry for the saddle on that bike. And yes, I know I still owe you all many beers!!!

DECLARATION

I hereby declare that the following thesis is based on the results of investigations conducted by myself, and that this thesis is of my own composition. This thesis has not, in whole or part, been previously presented for a higher degree. Work other than my own is clearly indicated in the text by reference to the relevant researchers or the publications.

Andrew Philp

The material contained within this thesis represents original work written by the author. The following published communications have been due to work contained within this thesis.

Philp, A., Macdonald, A.L., and Watt, P.W. (2005) Lactate – a signal coordinating cell and systemic function. *J. Exp. Biol.* 208(Pt 24): 4561-4575.

Philp, A., Macdonald, A.L., Harrison, M., Bone, A.J. and Watt, P.W. (2005) Arterialised, venous and fingertip capillary blood glucose disposal following a single bout of exercise and carbohydrate ingestion. *J.Physiol. 565P PC18*

Philp, A., Macdonald, A.L., Carter, H., Watt, P.W. and Pringle, J.S. (2008) Maximal Lactate Steady State as a training stimulus. *Int. J. Sports. Med*. 29(6): 475-479.

CHAPTER I - INTRODUCTION

The term metabolism describes the sum of all the reactions within the body that transduce chemical energy for cellular processes from ingested substrate stores (Berg et al., 2007). The most prevalent of these reactions in skeletal muscle is the catabolism of dietary lipid, carbohydrate or protein stores to generate adenosine tri-phosphate (ATP), the energy unit of the cell. During endurance exercise, the majority of energy is derived from lipid and carbohydrate stores. This process is tightly regulated, with a number of feedback and feed-forward mechanisms ensuring that the cell can switch between lipid and carbohydrate metabolism when required. Further, recent research has identified a number of substrate sensitive transcription factors, transcriptional co-activators and nuclear receptors which add further levels in which skeletal muscle metabolism is regulated (Berg et al., 2007).

During exercise, the key determinant in the selection of carbohydrate or lipid metabolism as the preferential fuel source is the intensity (how hard) and the duration (how long) of the muscle contraction. As a 6-carbon molecule, glucose, the principal dietary carbohydrate, is readily metabolised without the requirement of oxygen, to pyruvate during a ten step enzyme process termed glycolysis (Brooks, 1999). The formation of pyruvate is fundamental to the progression of metabolism, as two reactions stem from this metabolite. Firstly due to the generation of lactate, via the enzyme lactate dehydrogenase (LDH) and secondly through the production of acetyl-CoA via pyruvate carboxylation within the pyruvate dehydrogenase complex (PDH). The latter is particularly important as Acetyl-CoA, following conversion to citrate forms the principal

donor for the tricarboxylic acid cycle (TCA) which initiates the cycling of carbon substrate to generate electrons (NAD and FAD) for the final stages of oxidative phosphorylation and the synthesis of ATP (Brooks, 1999, Berg et al., 2007)(Figure 1.1).

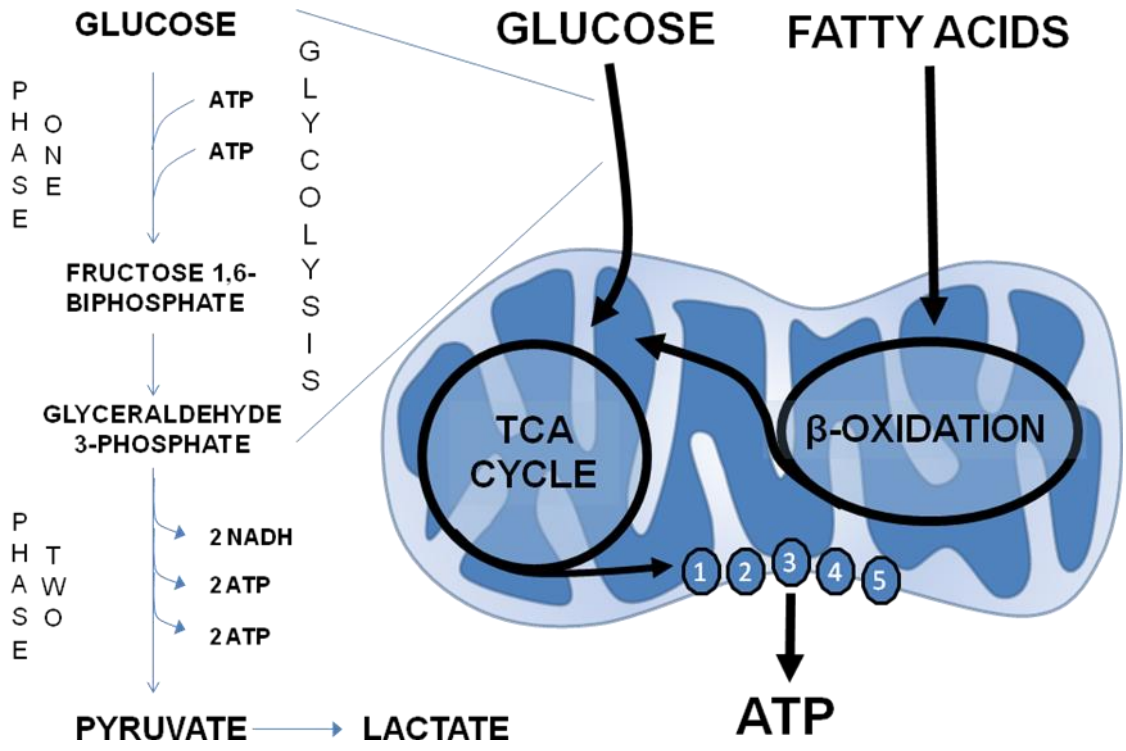

Figure 1.1. The interaction of glucose and fatty acid metabolic pathways in the intracellular generation of ATP

The uptake of glucose into the cell in skeletal muscle is facilitated by two membrane transport proteins, GLUT1 and GLUT4. As the principle glucose transporter in adult skeletal muscle, the regulation of GLUT4 has received substantial examination in recent years. GLUT4 has been shown to be regulated by both insulin and factors derived directly from muscle contraction (Brooks, 1999). Once glucose has passed the cell membrane it is phosphorylated by hexokinase and enters the cytosolic compartment upon

which glycolysis is initiated. Glycolysis can be viewed as two sections. The initial three steps are known as the ‘preparatory phase’ in which energy is consumed during the breakdown of the 6 carbon bond glucose molecule to two, 3 carbon molecule, whilst the second phase is known as the ‘replenishment phase’ whereby 2 molecules of ATP is generated during the conversion of glucose to pyruvate (Brooks, 1999). The first step of the pathway is the degradation of glucose to glucose-6-phosphate by the enzyme Hexokinase, which is also the first rate limiting step (i.e. irreversible) of glycolysis. Hexokinase phosphorylates glucose, utilizing ATP in the process forming glucose-6-phosphate. This is important in the progression of glycolysis as it retains a cellular concentration gradient (high to low from circulation to cytosol). To the present day, glycolysis is referred to in recognised, established textbooks, as aerobic and anaerobic due to the fact that the degradation of glucose to pyruvate can continue in the absence of oxygen, with 2 molecules of ATP and NADH formed during the reaction. With regard to exercising skeletal muscle, the term anaerobic glycolysis is somewhat confusing in this context as research has shown that in mammalian tissue, glycolysis is active when sufficient oxygen is available. The term anaerobic glycolysis is also instrumental in the labelling of lactate as a product of insufficient oxygen availability and surplus pyruvate formation. In fact lactate is formed during periods of metabolic homeostasis such as basal metabolism, with lactate levels exceeding pyruvate by a factor of 10:1 (Brooks, 1999).

The ability to sustain prolonged endurance exercise ultimately depends on the body providing sufficient oxygen to muscle to allow aerobic re-phosphorylation of ADP to ATP. Therefore, when examining endurance exercise at its broadest

context, a combination of factors appears to influence the aerobic capacity of an individual (Coyle, 1995, Bassett and Howley, 2000, Joyner and Coyle, 2008). The physiological role of lactic acid has been a source of investigation for the past two centuries. To this day, the exact purpose, positive or negative, of lactate production by the exercising muscle is unknown. During the past two hundred years, lactate has been viewed as a muscular energy source, a waste product which causes muscle fatigue and a cellular signal for muscular adaptation (Gladden, 2004). Whilst the positive roles for lactate are becoming increasingly apparent, the dogma of lactate acting as a muscular waste product remains commonly held by coaches, athletes and the general public. The reason for this is that research during the early to mid part of this century, by a number of highly regarded physiologists and biochemists, placed lactate as a muscular waste product which caused muscle to fatigue. As an example, the following is taken from a review article published in 1930 (Bancroft and Bancroft, 1930).

'When a muscle contracts, a certain amount of lactic acid is liberated in it, in proportion to the amount of work done by the muscle. Very considerable amounts may be set free in the muscles of a man during hard work. An ounce of it may easily be formed when a man runs 100 yards as fast as he can. Now lactic acid is a pretty strong reagent, and there is no doubt today that fatigue is due mainly to its formation in the inside of muscle-fibres. The function of oxygen is to remove the acid, once it has been formed.' (Bancroft and Bancroft, 1930)

It is important to note at this point that definitive experiments, designed to clarify lactate's specific role in muscle fatigue remain to be conducted. Accordingly, the literature is divided as to whether the release of lactate during muscle contraction is a positive or negative factor (Lindinger and Heigenhauser, 2008,

Boning and Maassen, 2008). Whilst the majority of this chapter will discuss the role of lactate during prolonged, sub-maximal exercise, it will also examine the regulation of substrate metabolism at the systemic and cellular level and discuss alternative hypotheses of exercise induced fatigue, independent of lactate release.

1.2. Physiological mechanisms of endurance performance

The underlying physiological mechanisms which regulate sub-maximal endurance performance are detailed in Figure 1.2. (Joyner and Coyle, 2008).

Figure 1.2. The physiological factors limiting endurance performance (Coyle and Joyner, 2008)

Ultimately, it is the total sum of a number of factors, termed morphological components, which allow the production of power or velocity. Important factors which have classically been suggested to regulate endurance performance are

the balance between aerobic and anaerobic energy provision, the individual maximal oxygen consumption ($\dot{V}O_2$max) and the power produced in relation to lactate turnover (i.e. the lactate threshold - LT).

Whilst $\dot{V}O_2$max (the maximal rate of oxygen uptake) and the power at the lactate threshold (the first and sustained increase in blood lactate concentrations from baseline during incremental exercise) are positive determinants of endurance performance, the control of substrate metabolism and the onset of fatigue are regulators of exercise sustainability.

1.3. The importance of oxygen uptake for endurance performance

Of all the measurements of aerobic capacity that are presently used in exercise science, the $\dot{V}O_2$max concept is the most established and yet still contested of all physiological assessment parameters (Levine, 2008, Noakes et al., 2001). The concept of $\dot{V}O_2$max is based on the belief that there exists a finite rate of maximal oxygen transport from the environment to the mitochondria to support oxidative production of ATP to perform physical work (Hill et al., 1924a, Hill et al., 1924c, Hill et al., 1924b). The classical view of $\dot{V}O_2$max is that oxygen utilization in skeletal muscle is limited by the ability of the cardiovascular apparatus to deliver oxygen. This hypothesis is supported by research showing that artificially increasing (Ekblom et al., 1972a, Ekblom et al., 1972b, Buick et al., 1980) or reducing (Ekblom et al., 1972a, Ekblom et al., 1972b, Levine et al., 1996) oxygen delivery alters $\dot{V}O_2$max.

Recently it has been contested whether $\dot{V}O_2$max really exists and how relevant this marker is for the assessment of individual aerobic capacity. The basis of the argument centres on one of the principal observations of Hill and colleagues early work (Hill et al., 1924a). The authors identified that on the attainment of maximal exercise intensities there was a plateau effect in the $\dot{V}O_2$ response, which demarcated the maximal rate of O_2 consumption and therefore the attainment of $\dot{V}O_2$max (Hill et al., 1924a, Hill et al., 1924c, Hill et al., 1924b). The second observation of Hill et al (Hill et al., 1924a, Hill et al., 1924c, Hill et al., 1924b) was that when the plateau was achieved, exercise could still be maintained for an additional time period (~2-3 minutes) however there were no further increases in oxygen consumption.

Professor Tim Noakes (Noakes et al., 2001, Noakes, 2007, St Clair Gibson et al., 2003, St Clair Gibson et al., 2006) has for a number of years suggested that it is peripheral limitations during exercise such as local skeletal muscle fatigue which cause exercise to terminate during incremental exercise to exhaustion, not central, cardiovascular limitations as suggested in Hill's original model. In his challenging of the $\dot{V}O_2$max paradigm, Noakes has developed his own 'Central governor' hypothesis which integrates peripheral and central signals to regulate exercise sustainability (Noakes et al., 2001). Conceptually, the hypotheses presented by Hill et al (Hill et al., 1924a, Hill et al., 1924c, Hill et al., 1924b) and Noakes (Noakes et al., 2001) are based upon the same phenomenon and they both acknowledge the influence of peripheral and central factors in determining physiological responses to exercise. The contrast in their interpretation is that Hill and colleagues suggested that the limitation to exercise performance comes from oxygen delivery limitations, whereas Noakes (Noakes

et al., 2001) suggests that local signals in skeletal muscle (local governor) sends sensory feedback to the brain (central governor) which regulates exercise sustainability.

$\dot{V}O_2$max has been used for many years as a measurement tool for assessing aerobic capacity in sedentary, active and elite athletic performance (Wasserman et al., 1990). Moreover $\dot{V}O_2$max, or an estimated form of $\dot{V}O_2$max, has also been suggested as an effective measurement for chronic disease states such as chronic obstructive pulmonary disease (COPD), myocardial infarction or type 2 diabetes (Levine, 2008). Indeed, in sedentary and moderately trained individuals $\dot{V}O_2$max is a good indicator of endurance performance, and the speed obtained at $\dot{V}O_2$max provides a good correlate of time trial performance (Jones and Carter, 2000). However, amongst elite endurance athletes, in which $\dot{V}O_2$max values are similar, a number of other factors, mainly sub-maximal, determine endurance performance (Coyle, 1995, Joyner and Coyle, 2008).

1.4. Exercise efficiency is a key determinant of endurance capacity and performance.

Whilst the capacity to extract oxygen from the environment and deliver it to working skeletal muscle is a key determinant of endurance performance, the ability to sustain a fractional load of individual maximal oxygen uptake (i.e. % $\dot{V}O_2$max) as well as the efficiency or economy of movement are two further, oxygen related, determinants of endurance performance (Joyner and Coyle, 2008). Conley and Krahenbuhl (1988) were amongst the first to demonstrate

that athletes with similar $\dot{V}O_2$max values had significantly different economy (usually defined as the oxygen cost of exercise at a given intensity) values during running, which accounted for vast differences in running performance time (Conley and Krahenbuhl, 1980). This observation has become even more prevalent as recent research has shown that eastern African runners have greater exercise economy than European counterparts and that this, in part, explains the superior performance of these individuals (Saltin et al., 1995, Foster and Lucia, 2007)

The standard assessment of running economy involves a relatively simple measurement approach. This requires an individual to exercise at a sub-maximal exercise intensity (below the ventilatory threshold) until a steady state in the $\dot{V}O_2$ response is observed. Exercise economy is then taken over a period (usually 5-10 minutes) with the $\dot{V}O_2$ for the given work-rate measured. For endurance athletes, running economy is usually expressed at six minute mile pace, which corresponds to 268 $m \cdot min^{-1}$ or 4.47 $m \cdot s^{-1}$ (Foster and Lucia, 2007). The lowest value reported in the literature at this intensity is 39.0 $ml \cdot kg \cdot min^{-1}$ by an individual with a 1500m personal best of 3:35 (Foster and Lucia, 2007).

1.5. Systemic lactate appearance and endurance performance.

The increase of lactate in the bloodstream is the result of factors affecting production and removal, which ultimately provides a measurement of lactate turnover. The predominant site of lactate production in humans during exercise is skeletal muscle, whilst removal can include skeletal muscle, the bloodstream and a variety of organs including the heart, liver and lungs. Donovan and

Brooks (1983) demonstrated clearly, in exercising rats, that lactate appearance and disappearance were linearly related to arterial lactate concentrations during graded exercise, and that both processes increased in accordance with exercise oxygen consumption (Donovan and Brooks, 1983). Importantly, Donovan and Brooks (1983) also demonstrated that at sub-maximal exercise intensities (<50% $\dot{V}O_2$max) there was a balance in lactate appearance and disappearance that resulted in a steady state of lactate turnover (i.e. equal net lactate turnover).

During the 1960-1970's, researchers observed that during incremental exercise, beyond a certain exercise intensity, there was a clear increase in systemic lactate concentrations. Coupled to this increase was an elevation in minute ventilation and $\dot{V}CO_2$ appearance (Davis, 1985). Based on the observations by Hill and colleagues (Hill et al., 1924a) and the establishment of the oxygen debt hypothesis (Hill et al., 1950), the increase in systemic lactate was interpreted to be due to local hypoxia and as such represented increased anaerobic glycolysis (Davis, 1985). The authors termed this transition the anaerobic threshold (AT) and this marker was accepted into mainstream exercise physiology as a valuable marker of exercise capacity (Davis, 1985). Unfortunately, the designation and widespread acceptance of the AT is a principal reason why lactate is commonly associated with oxygen lack and is associated as a principal cause of fatigue during strenuous exercise (Brooks, 1986).

1.6. The maximal lactate steady state and endurance performance.

The MLSS was first conceptualized in the study of Stegmann and Kindermann (Stegmann and Kindermann, 1982) who examined prolonged exercise at a pre-determined anaerobic threshold or a fixed lactate concentration of 4mM. The authors hypothesized that exercise above the anaerobic threshold and exceeding 4 $mmol \cdot l^{-1}$ in systemic lactate concentration resulted in rapid fatigue during exercise, and as such represented a critical exercise intensity for endurance performance (Stegmann and Kindermann, 1982). The term maximal lactate steady state (MLSS) was first introduced by Beneke and colleagues (Beneke, 1995). Beneke introduced and validated a standardized protocol for measuring the MLSS which consisted of a series of 30min constant load tests on separate laboratory visits. It was found that approximately 3-5 experimental test sessions could accurately be used to ascertain the MLSS. Beneke (1995) defined the maximal lactate steady state (MLSS) as the highest running speed or power output at which blood lactate concentrations remain stable between 10 and 30 minutes of constant load exercise (Beneke, 1995).

Since the initial observations by Stegmann and Kindermann (1982) the MLSS is often associated with the anaerobic threshold (AT) and OBLA (Billat et al., 2003). However, contemporary research would suggest that the AT and OBLA are inaccurate markers to assess physiological responses to sub-maximal exercise (Billat et al., 2003). The AT concept encompasses a number of physiological phenomena, and is based on the premise that systemic lactate concentrations increase in parallel to respiratory alterations (Brooks, 1985). Current research would now separate the AT into two independent phenomena,

namely the first, sustained increase in lactate during progressive exercise being termed the lactate threshold (LT), whilst the first and sustained increase in minute ventilation or $\dot{V}CO_2/\dot{V}O_2$ would be termed the ventilatory threshold (VT) (Jones and Carter, 2000). Further, both of these intensities are now widely regarded to occur at power outputs below a 4mM lactate reference (Billat et al., 2003) so dissociating LT/VT from OBLA, finally, there is also recognized dissociation between the LT and VT separating lactate appearance from alterations in ventilatory drive (Jones and Carter, 2000).

The MLSS is regarded as the gold standard to assess individual endurance capacity for events lasting one hour in duration (Jones and Carter, 2000, Billat et al., 2003). Importantly for physiological assessment, the exercise intensity maintained at MLSS demonstrates a strong correlation with LT, 8km, 10km (Jones and Doust, 1998) and marathon performance times (Billat et al., 2003)(Figure 1.3).

Figure 1.3. The correlation between the running speed at the MLSS and 8km performance and the Lactate Threshold.

The main criticism of the MLSS is the laborious nature of the Beneke protocol for determining the power output or speed at MLSS (Billat et al., 2003). Accordingly, the majority of research regarding the MLSS has focused on the question as to whether the MLSS can be estimated from incremental exercise protocols (Swensen et al., 1999, Harnish et al., 2001, Snyder et al., 1994, Palmer et al., 1999, Laplaud et al., 2006, Kuphal et al., 2004, Beneke, 2003b, Beneke, 2003a, Billat et al., 1994, Billat et al., 1995). It has been suggested that heart rate (Vobejda et al., 2006), LT (Faude et al., 2009), VT (Yamamoto et al., 1991, Van Schuylenbergh et al., 2004), the lactate minimum (Sotero et al., 2009, Jones and Doust, 1998), Critical Power (Smith and Jones, 2001, Pringle and Jones, 2002, Dekerle et al., 2003), OBLA (Denadai et al., 2005, Figueira et al., 2008) can all be used to estimate the MLSS. However, despite a great deal of attention and research time, currently the Beneke model is still regarded as the standard measurement tool for accurately attaining the MLSS (Billat et al., 2003, Figueira et al., 2008).

The sensitivity of the MLSS to endurance training has also been examined, either through using the MLSS as an exercise stimulus (Billat et al., 2004) or examining the effect of standardized training on the lactate concentration or the power output at MLSS (Carter et al., 2000). The lactate concentration at MLSS has been shown to vary with age in a fibre-type dependant manner (Mattern et al., 2003) and with the mode of exercise used (Beneke et al., 2001), suggesting the importance of lactate production and removal capacities in the maintenance of MLSS. Further, cross sectional comparisons of sedentary, trained and elite

trained individuals have also been made, further demonstrating the sensitivity of the MLSS to training status (Hoogeveen et al., 1997, Almarwaey et al., 2004).

There is, however, a dearth in the MLSS literature examining the physiological mechanisms which regulate exercise at and above the MLSS (Billat et al., 2003). What is known is that (1) the exercise duration at the MLSS appears to be ~60mins for both cycling and running exercise (Lajoie et al., 2000, Fontana et al., 2009), (2) other metabolites associated with fatigue increase during exercise at the MLSS (Baron et al., 2003) and (3) that fatigue at the MLSS does not appear to correlate with an increase in systemic lactate concentrations at the MLSS (Baron et al., 2008) (Figure 1.4).

Figure 1.4. Systemic lactate concentrations demarcating exercise intensity transition zones.

The physiological importance of the MLSS is that it represents the highest exercise intensity at which a balance exists between systemic lactate appearance and disappearance, which in turn reflects rates of cellular utilization and recycling of lactate. When exercise intensity exceeds the MLSS, an increase in systemic lactate and reduced time to volitional exhaustion is observed. It therefore appears that lactate turnover (i.e. the process of lactate appearance and disappearance during exercise) is either directly, or in part, associated with first, the regulation and sustainability of exercise and second, the development of fatigue during prolonged endurance exercise in humans (Billat et al., 2003). Previous research, based on whole system physiological responses during exercise, places the MLSS as the transition point from the heavy to severe exercise domains (Figure 1.4). It still remains to be determined whether the MLSS represents a clear physiological threshold, and the relevance of this transition to exercise (Billat et al., 2003).

The following review of literature will seek to detail the interaction between glucose, lactate and pyruvate during prolonged exercise and detail how this interaction can be used to clearly assess measure and define human response to exercise.

CHAPTER II – REVIEW OF LITERATURE

2.1. From milk to muscle – A brief history of lactate metabolism

Lactic acid has been a source of intrigue and frustration for biochemists, exercise physiologists and the athletic population for over 200 years (Gladden, 2008). From its early discovery in sour milk, which helped to give the acid its name, to its modern central role in substrate metabolism, lactate certainly has shown an unrivalled diversity in the manner by which it influences mammalian function (Gladden, 2008). In 1950, von Muralt defined the investigation of muscle chemistry to be demarcated by four distinct eras: pre-lactic acid, lactic acid, phosphorylation and myosin (Holten et al., 1971). Gladden (2004) recently introduced contemporary lactate understanding with what he termed the lactate paradigm for the third millennium (Gladden, 2004). This concept sees lactate as a positive, functional metabolite, supporting exercise function, shuttling between sites of production and oxidation whilst assisting muscle in contractile function (Gladden, 2004).

Lactic acid was first discovered in 1780 by the Swedish chemist Carl Wilhelm Scheele (Holten et al., 1971), who found that milk samples, when turning sour, also developed a high acidity. The substance identified, which turned out to be 2-hydroxypropanoic acid, soon became known as lactic acid due to lactic meaning of or related to milk. In 1808 the Swedish chemist Berzelius noted that the skeletal muscle of deer contained a particularly high acidity (Berzelius, 1808, Holten et al., 1971). Further findings suggested that this acidity was caused by lactic acid, and was present at high concentrations in both blood and

muscle. By 1833, pure samples of lactic acid had been isolated and further investigation identified that two optimal isomers of lactate existed – D and L (Holten et al., 1971).

Early investigations failed to expand or explain Berzelius's observations in muscle, and instead focused on lactic acid formation during fermentation reactions (Araki, 1891). In 1845, von Helmholtz suggested that the production of lactate might derive from glycogen stores, whilst Du Bois-Reymond (Holten et al., 1971) linked muscle contraction to the development of acidity, and the development of acidity to be caused by the accumulation of lactic acid. Heidenhain (1864) reported a positive correlation between the intensity of contraction and the increase in lactic acid (Araki, 1891, Holten et al., 1971).

The principal methodological milestone of this era was achieved in 1907 by the landmark experiments of Fletcher and Hopkins in isolated amphibian muscle (Fletcher and Hopkins, 1907). Through progressive investigations, the authors identified that (1) lactic acid was low in resting muscle, (2) it increased when this muscle was denied oxygen, (3) lactic acid increased when a muscle was electrically stimulated to contract, and (4) accumulated lactate disappeared when oxygen was introduced to the muscle preparation, suggesting a central role for oxygen in the appearance and disappearance of lactic acid (Fletcher and Hopkins, 1907).

2.2. The revolution in muscle physiology

During the 1920's, work by three predominant research groups, A.V. Hill's in London, the Heidelberg group of Otto Meyerhoff, and Dill and Margaria's group at Harvard, provided much of the basis for our understanding of lactate metabolism in exercise physiology. In 1923, Hill and Meyerhof combined their research observations and many of the accepted or hypothesized theories at the time in a historical review article (Hill and Meyerhof, 1923). The two main theoretical constructs to emerge from this paper were the identification and naming of the 'lactic-acid-cycle' (describing the processes utilizing the cyclical conversion of glycogen to lactic acid back to glycogen) and secondly the recognition that 'two' distinct pathways supplied the energy required for muscle contraction, which were deemed aerobic (in the presence) and anaerobic (in the absence) of oxygen (Hill and Meyerhof, 1923). The working hypothesis for Hill and Meyerhoff's research at this time was that the hexosediphosphate compound discovered by Emben and Laquer in 1914, which they termed lactacidogen, was the source of energy in muscle contraction (Embden and Laquer, 1914, Embden and Laquer, 1917).

During the following decade, additional steps in the glycolysis pathway were discovered and it was demonstrated that energy provision for muscular contraction resulted from the degradation of high energy phosphate compounds (Gladden, 2008). This discovery led to the question of lactate's exact role in locomotion. Lactate was not the energy provider that Hill and Meyerhoff had proposed, however it was associated with exercise to exhaustion in both *in vitro* and *in vivo* experimentation.

Whilst Meyerhoff's research concerned the lactic acid cycle on non-circulated amphibian hemicorpus preparations, Hill and colleagues (Hill et al., 1924a, Hill et al., 1924c, Hill et al., 1924b) subsequently sought to investigate this phenomenon in humans during exercise. From a series of experiments and observations, the authors determined the rise in lactic acid at the onset of exercise to be as a direct result of O_2 lack (hypoxia) in exercising skeletal muscle. The 'oxygen debt model' that Hill and colleagues postulated, supported with subsequent work from Hill's laboratory (Hill et al., 1950), became the primary explanation for the increased appearance of lactic acid during exercise and ensuing fatigue. The hypothesis proposed that during recovery, one-fifth of the lactate was oxidised to provide energy for the conversion of the remaining four-fifths of lactate back to glycogen (Hill, 1924).

Subsequent recognition of these researcher's contributions (Hill and Meyerhoff were jointly awarded the Noble Prize for science in 1922), saw the O_2 debt hypothesis accepted as a leading theory in the physiological understanding of prolonged human exercise, whilst providing the paradigm for the body of further human research that ensued (Hill et al., 1950).

2.3. Challenging a dogma

In many ways, as powerful as Hill and Meyerhoff's observations were in shaping future research into the causes, mechanisms and benefits of exercise, they are the primary source of lactate's labelling as a metabolic waste product serving to compromise exercise performance, one which it has struggled to shake off over the following 80 years (Brooks, 2002b). No one at this time

would have conceived that lactate could play a beneficial role in exercise performance, or prove to be such a mobile metabolic intermediate. There were, however, interspersed in his period, experiments which would slowly unravel lactates role in the O_2 debt hypothesis (Gladden, 2004).

The first of such experiments were conducted by Margaria et al., (1933). In a series of experiments on humans during short duration, high intensity exercise, they managed to define two distinct phases of the post exercise recovery response (Margaria et al., 1933). Importantly they noted that the oxygen and lactate kinetic response in the immediate post exercise period was dissociated, and therefore could not be related, whilst during the second phase, lactate and oxygen recovery seemed to coincide (Margaria et al., 1933, Margaria, 1934, Margaria et al., 1971b, Margaria et al., 1971a). Subsequently, Margaria et al., (1933) termed the first phase alactacid (i.e. not associated with lactate metabolism) and the second lactacid, as this phase was suggested to be involved in the conversion of lactate to glycogen (Margaria et al., 1933, Margaria, 1934, Margaria et al., 1971b, Margaria et al., 1971a).

Apart from research such as that generated by Cori's laboratory, which demonstrated that lactate could be converted back to glucose in the liver (Cori et al., 1939, Cori and Shine, 1935), research during the next twenty years sought to prove lactate as the cause of fatigue, rather than to question its function (Gladden, 2004).

Clearly research at this time had begun to dissect that lactate and oxygen lack during and in recovery from exercise were more a coincidence than causative.

There was still a body of research, however, supporting the notion of lactate as an anaerobic waste product, with lactate acidosis (the combined effect of lactate and hydrogen ion release) suggested as a principal cause of exercise induced fatigue (Davis, 1985).

2.4. Is lactate really an anaerobic waste product?

During the 1980's, new research groups set out to question whether or not lactate was a waste product. Initially, work by Jobsis and Stainsby (1968), and later by Connet et al., (1986) demonstrated that stimulated canine muscle was capable of producing and oxidising lactate at conditions equivalent to moderate intensity exercise, at which there was, seemingly, an adequate supply of oxygen (Jobsis and Stainsby, 1968, Connett et al., 1986). In light of this, Brooks (1986) postulated that for oxygen lack (anaerobiosis) to be the primary cause of lactate accumulation, muscle anoxia must exist, since this was thought to be the stimulus for lactate production (Brooks, 1985, Brooks, 1986a, Brooks, 1986b). Numerous research studies had previously demonstrated that at exercise intensities of 50 - 75% $\dot{V}O_2$max, where the AT supposedly occurred, sufficient reserves of cardiac output, localised blood flow and lactate and glucose arterial-venous differences existed for muscle to remain suitably perfused for aerobic metabolism to continue, thus providing conflicting information to that of the AT theoretical construct (Brooks, 1985, Brooks, 1986a, Brooks, 1986b).

Richardson et al., (1998) utilised phosphorous magnetic resonance spectroscopy (MRS) and myoglobin saturation, as measured by ^{1}H nuclear

MRS, to address whether lactate increase during progressive exercise to exhaustion was due to muscle hypoxia (Richardson et al., 1998). They observed that net blood lactate efflux was unrelated to intracellular pO_2 across work intensities, but linearly related to O_2 consumption and intracellular pH. Therefore the data provided by Richardson et al., (1998) supports the notion that lactate efflux during exercise is unrelated to muscle cytoplasmic pO_2, effectively dissociating lactate production and hypoxia (Richardson et al., 1998).

Comparative examination, across the animal kingdom of the glycolytic pathway has provided evidence that anaerobic conditions are not essential for lactate to be produced, demonstrating that energy systems work in unison as opposed to switching on and off, whilst duly confirming the dissociation between lactate and hypoxic or anoxic conditions. The tailshaker muscle of the Western diamondback rattlesnake (*Crotalus atrox*) has provided a model which clearly demonstrates that aerobic metabolism can meet a high ATP demand. Species such as the rattlesnake are able to alter the energy requirement of muscle contraction so that glycolysis may continue (Figure 2.1). Tailshaker muscles are capable of sustaining high frequency contractions in the region of 20 – 100Hz for several hours with an ATP cost per twitch of 0.015 mM ATP per gram of muscle (Conley and Lindstedt, 1996). Utilising the same model, this time in ischemia and normoxic situations, Kemper et al., (2001) demonstrated that elevated rates of glycolysis could happen independently of O_2 levels (Kemper et al., 2001). Such muscle was capable of exercising without fatigue due to high blood flow levels allowing the rapid turnover of H^+ and lactate (and presumably other metabolites that might themselves be involved in a fatigue process) within the cells. Recent research suggests that mechanical trade-offs between twitch

tension and duration and between joint force and displacement explain how the tailshaker muscle can alter rattling frequency rates without increasing the metabolic cost of activity (Moon et al., 2002).

Figure 2.1. The tailshaker muscle of the Western diamondback rattlesnake (*Crotalus atrox*) can maintain high frequency contraction for long durations due to elevated glycolytic ATP supply (Conley and Lindstedt, 1996).

This data allows for two considerations. Firstly it allows for the acceptance that lactate is not only produced due to anoxic or hypoxic conditions, rather it is a metabolite produced during adequate oxygen provision. Secondly, aerobic ATP provision is a highly adaptable process, with skeletal muscle possessing an inherent ability to adapt to the energy requirements of the organism. It appears that many animal species are able to minimise the cost of muscle contraction so that cellular ATP production can meet ATP demand and sustain high contractile rates (Conley et al., 2001) with lactate formed as an integral part of this working system, not as an end product *per se*.

2.5. A metabolite on the move - membrane facilitated lactate transport

During conditions of lactate production, at rest and during sub-maximal exercise, substrate concentrations support the conversion of pyruvate to lactate via the lactate dehydrogenase (LDH) reaction. Until relatively recently, our understanding, was that lactate moved from the cellular compartment, to the blood via simple diffusion. Increased muscle lactate concentrations were deemed a consequence of increased glycolytic flux rates, with cellular function inhibited when lactate was unable to leave the cytosolic compartment (Brooks, 1986a).

This understanding began to change following initial observations in rodent studies by Donovan and Brooks (1983), which demonstrated that endurance training reduced post exercise lactate concentrations by enhancing lactate clearance. This led the authors to hypothesise that the major fate of lactate during or following exercise was probably via oxidation (Donovan and Brooks, 1983). Further research demonstrated that lactate transport was sensitive to pH, specific transport inhibitors, and temperature (Juel and Wibrand, 1989, Watt et al., 1988, Roth and Brooks, 1990a, Roth and Brooks, 1990b).

To directly measure lactate kinetics in humans, Mazzeo et al., (1986) used the stable isotope tracer [1-^{13}C] lactate to demonstrate that the rate of lactate disposal (R_d) was directly related to metabolic clearance rate (MCR). That oxidation, as determined by the appearance of ^{13}C enrichment in CO_2, was the major fate of lactate during exercise, and subsequently to this, that the interpretation of lactate kinetics by way of concentrations was inappropriate, as

circulatory endpoint values could not reflect lactate turnover in muscle (Rate of production - Rate of removal) (Mazzeo et al., 1986). Donovan's findings were supported in humans (Bergman et al., 1999), whilst subsequent animal research in giant sarcolemmal vesicle and perfused hindlimb preparations added support to a carrier mediated process for lactate transport in and out of skeletal muscle, as well as the stimulatory effects of contraction, pH and blood flow on both processes (Juel and Wibrand, 1989, Watt et al., 1994, Gladden et al., 1995).

The process of facilitated lactate transport across the cell membrane was initially thought to be mediated by pH diffusion gradients, predominantly regulated by alterations in redox balance (Brooks, 1999). However, Deuticke et al., (1982) were the first to demonstrate in erythrocytes that lactate transport across the cell membrane occurred via three distinct pathways; firstly, via carrier mediated transport by an H^+ coupled monocarboxylate tranporter (MCT), secondly, by exchange with inorganic anions via the band 3 protein, and finally, via passive diffusion across the lipid bilayer. Interestingly, Deuticke and co-workers (1982) observed that under physiological conditions, approximately 90% of lactate transport is mediated by the MCT pathway (Deuticke, 1982, Deuticke et al., 1982).

2.6. Characterization of the mammalian monocarboxylate transport (MCT) protein family

Poole and Halestrap (1993) identified that the H(+)-monocarboxylate transporter of the erythrocyte membrane, was capable of transporting L-lactate

and a wide range of other aliphatic monocarboxylates, such as pyruvate and the ketone bodies acetoacetate and beta-hydroxybutyrate (Poole and Halestrap, 1993). Further, these authors observed that the transport protein is inhibited by α-cyano-4-hydroxycinnamate derivatives (CINN), the stilbene disulfonates 4,4'-diisothiocyanatostilbene-2,2'disulfonic acid (DIDS) and phloretin. On the basis of purification and specific labelling experiments, the MCT was identified as a protein in the region of 35-50 kDa, however at this time the molecular characteristics of the erythrocyte MCT were unknown (Poole and Halestrap, 1993).

The first development in the characterization of the mammalian MCT came when Kim and coworkers (Kim et al., 1992) cloned a membrane protein from met-18b-2 cells (Faust and Krieger, 1987) which they termed Mev, due to its ability to transport the G-carbon branched dihydroxy-monocarboxylate mevalonate (Kim et al., 1992). When Garcia et al., (1994) cloned and sequenced the crosshybridizing cDNA from wild-type CHO cells they found that wild-type Mev differed from the met-l 8b-2 cell protein at one nucleotide, which changed a codon in membranespanning region 10 from phenylalanine (wild-type) to cysteine (Mev). The wild-type cDNA did not elicit increased mevalonate transport in transfected CHO cells leading to Kim et al., (1992) suggesting that the wild-type Mev transported a substance other than mevalonate (Garcia et al., 1994).

Kim et al (1992) were unable to identify the endogenous substrate for the wild-type Mev transporter. However, subsequent work from Garcia et al., (1994) demonstrated that the wild-type version of Mev is an MCT that exhibits the

classic properties of the previously described erythrocyte MCT. Accordingly, Garcia et al (1994) designated this protein as MCT1.

Following the identification of MCT1, a whole family of MCT transporter proteins have been identified (Juel and Halestrap, 1999, Halestrap and Meredith, 2004). Garcia and colleagues (Garcia et al., 1994) cloned, sequenced and expressed a second MCT isoform, which shared 60% homology with MCT1 which they termed MCT2. MCT2 has since been cloned and sequenced in rat, mouse and human tissue (Jackson et al., 1997) however the exact physiological role for this transporter is currently unknown (Hashimoto et al., 2006).

The next MCT to be cloned was isolated from a chicken retinal pigment epithelium cDNA library and was named MCT3 as it was distinctly different from MCT1 and MCT2, sharing 43 and 45% homology respectively (Philp et al., 1995). Following a database screen for expression sequence tags (dbEST) for fragments of MCT-like sequences, Price et al (1998) identified four new MCT isoforms, exhibiting 30-60% similarity with MCT1, and accordingly named these isoforms MCT4-MCT7 (Price et al., 1998). The MCT family now comprises of 14 members, however conservation of sequence between isoforms is greatest for MCT1-MCT4 (>50%), suggesting close substrate specificity for these isoforms (Halestrap and Meredith, 2004).

Each MCT transporter protein features twelve transmembrane-spanning (TM) helices with intracellular C- and N- termini and a large intracellular loop between segments 6 and 7 (Juel and Halestrap, 1999). It has been proposed that the two halves of the protein, TM helices 1-6 and TM helices 7-12 have distinctly

different physiological roles (Juel and Halestrap, 1999). The N- terminal domains may be important for energy coupling (e.g. via H^+ or Na^+ cotransport), membrane insertion, and/or correct structure maintenance (Juel and Halestrap, 1999). In contrast, the C- terminal domains may be important for the determination of substrate specificity. In fact, the conversion of Phe_{360} to Cys in TM segment 10 of CHO MCT1 changes the protein from a lactate/pyruvate transporter to a mevalonate transporter (Kim et al., 1992). Finally, the binding site on MCT1 for the pharmacological inhibitor DIDS is at or near the substrate binding site in the C-terminal domain of MCT1(Poole and Halestrap, 1993).

Expression studies and immunohistochemical analysis has identified that MCT1 and MCT4 are the major MCT isoforms expressed in skeletal muscle. Slow oxidative muscle, such as the rat soleus, was found to be rich in MCT1 expression, whereas muscle containing predominantly glycolytic fibres, such as the semitendinosus, contain little MCT1 (McCullagh et al., 1996). Further, MCT1 has been found to be highly expressed in cardiac muscle (Halestrap et al., 1997) supporting the notion that MCT1 facilitates the transport of lactate into the muscle where it can be oxidized as a fuel (Van Hall, 2000, Brooks, 2002b). The mechanism of transport involves an ordered, sequential mechanism, whereby a proton first binds to the transporter, followed by the lactate anion. This is then followed by the translocation of lactate and the proton across the membrane and their sequential release from the transporter on the other side of the plasma membrane (Juel and Halestrap, 1999). This process is freely reversible, however the rate-limiting step for net lactate flux appears to be the return of the carrier across the membrane, which is required to complete the translocation cycle (Juel and Halestrap, 1999). It has also been observed that at

physiological pH, a reduction in extracellular pH will increase transport, primarily through decreasing the K_m for lactate. Similarly, elevations in intracellular pH may act to increase the V_{max} of transport by increasing the rate at which the unloaded MCT re-orientates at the membrane (Juel and Halestrap, 1999).

2.7. Exercise increases skeletal muscle MCT and lactate dehydrogenase (LDH) protein content

Donovan and Brooks, (1983) were the first to demonstrate that muscle contraction improved the lactate clearance capacity of muscle (Donovan and Brooks, 1983). This anecdotal evidence suggested that contraction could directly affect MCT content and function. Indeed, prior to the identification of the skeletal muscle MCT's, both high intensity and endurance type training had been shown to increase the maximal rate of lactate transport into sarcolemmal vesicle preparations by 30-100% (McDermott and Bonen, 1993b, McDermott and Bonen, 1993a, Pilegaard et al., 1993).

Dubouchard et al., (2000) were the first to present evidence that endurance training could increase the skeletal muscle protein content of MCT1 and MCT4, with MCT1 appearing to have a greater sensitivity to the training period. Importantly, MCT1 was determined in sarcolemmal and mitochondria enriched muscle fractions, whereas MCT4 was only demonstrated in sarcolemmal fractions. Secondly, MCT4 showed a varied response to training, and also great inter-individual differences amongst the subject cohort. Finally, MCT1 correlated highly with MHC-I expression and the training induced increase in citrate synthase activity (Dubouchaud et al., 2000).

Whilst adaptation in the content and expression of MCT1 and MCT4 is principal in the modulation in skeletal muscle lactate transport capacity following endurance training, adaptation in lactate dehydrogenase (LDH) expression has also been suggested to be an important training induced adaptation (Van Hall, 2000) due to LDH's ability to control the formation of lactate and therefore directly regulating the turnover of cellular lactate (Van Hall, 2000).

LDH is a tetramer composed of either M (muscle) or H (Heart) isoforms which combine to form five isozymes (LDH_{1-5}). LDH_1 (H_4) and LDH_2 (H_3M) predominantly occur in the heart whereas LDH_4 (HM_3) and LDH_5 (M_4) are mostly found in muscle and liver (DeDuve et al., 1964). Heart LDH is maximally active at low concentrations of pyruvate and is strongly inhibited by excess pyruvate (DeDuve et al., 1964). In contrast, the M isoform, maintains its activity at relatively high pyruvate concentrations. DeDuve also suggests these differences are suggestive for specific roles for LDH in cardiac and skeletal muscle. In cardiac muscle, a steady state of substrate supply is required, maintained by the complete oxidation of pyruvate and lactate in the mitochondria (DeDuve et al., 1964). Therefore, the inhibition of LDH_1 by pyruvate favors this process. Conversely, skeletal muscle favours an intermittent supply of substrate, occurring sometimes in reduced oxygen situations. Glycolysis generates high concentrations of pyruvate and requires pyruvate reduction to lactate to allow this process to continue. Muscle LDH allows this process to take place, despite fluctuating levels of pyruvate as the lactate formed is shuttled to alternative tissue beds for subsequent oxidation (Van Hall, 2000).

With the increased knowledge of MCT facilitated lactate transport, evidence in support of the lactate shuttle hypothesis was becoming widespread. Brooks (1986) postulated the framework of the lactate shuttle hypothesis prior to the discoveries of MCT or their distribution (Figure 2.2) and gradually research confirmed his original concept. This lactate shuttle hypothesis proposed that lactate was able to transfer from its site of production (cytosol) to neighbouring cells and a variety of organs (e.g. liver, kidney and heart) where its oxidation or continued metabolism could occur. Of key importance to this hypothesis was the appreciation that for lactate shuttling to occur, as suggested, a cellular protein transport system would be implicated (Brooks, 1986a).

Figure 2.2. Cell to cell lactate shuttle as originally proposed by Brooks, 1986 (Adapted from Gladden, 2004)

The original lactate shuttle hypothesis has since seen a number of revisions with an intracellular component introduced (Figure 2.3) (Brooks, 2000). The extension to an intracellular shuttle system has not been without its controversy. The principle depends upon the presence of mitochondrial LDH (mLDH) for the re-conversion of lactate, once it enters the mitochondrion, to pyruvate and for mitochondrial located MCTs (Brooks, 2000). This component has been strongly challenged by two independent investigations (Rasmussen et al., 2002, Sahlin et al., 2002) The principle flaw to the Brooks model, detailed by these authors, was that lactate entering the mitochondria would create a futile cycle by which pyruvate is reduced to lactate in the mitochondria and vice versa in the cytosol. It was suggested that this would induce a situation compromising energy production as both the redox state of the cell and the required direction of substrate flow would be reversed.

This suggested scenario however seems unlikely. Firstly, in conversion of pyruvate to lactate, lactate accepts a H^+ ion from NADH, thereby allowing increased availability of NAD, and maintenance of the redox state of the cell. Secondly, within the intracellular model a futile cycle would not be formed, as lactate entering the mitochondria would be converted to pyruvate and oxidised. Lactate acts as an alternative pathway for substrate to enter the mitochondria, competing with pyruvate for MCT transport.

The intracellular shuttle (Figure 2.3) does not suggest that pyruvate is not present in the intracellular compartment, instead that the LDH conversion of lactate to pyruvate is more than a cytosolic reaction alone. Data provided by Laughlin et al., (Laughlin et al., 1993) utilising magnetic resonance

spectroscopy (MRS) in working canine hearts, has proven that infusion of ^{13}C-pyruvate labels cytosolic lactate and alanine pools, whereas ^{13}C-lactate did not label cytosolic pyruvate or alanine. However, the TCA cycle substrate, α-ketoglutarate was labelled, suggesting infused lactate by-passed the cytosolic LDH reaction and was converted to pyruvate in the mitochondria. Brooks questioned the methods used by the Rasmussen and Sahlin laboratories in obtaining mitochondria, suggesting that mLDH could easily have been lost during this subfractionation process and was the main reason for the discrepancies in results (Brooks, 2002a, Brooks, 2002b).

Figure 2.3. The intracellular lactate shuttle hypothesis added a mitochondrial lactate shuttle to the original cell to cell system (Adapted from Gladden, 2004).

Further, the controversy over mitochondrial located MCTs might have been resolved by two recent studies (Butz et al., 2004, Hashimoto et al., 2006,

Hashimoto et al., 2005) with the latter using immunohistochemical analysis in combination with confocal laser scanning microscopy (CLSM) to clearly demonstrate the co-localisation of MCT1 and cytochrome oxidase (COX) at both interfibrillar and subsarcolemmal cell domains. This data would indicate that MCTs and associated proteins are therefore positioned specifically to facilitate functions of the lactate shuttle system (Gladden, 2008).

2.8. Cellular sites of lactate shuttles

Lactate has been suggested to play an important role in cellular and organelle redox balance, a function demonstrated in the proposed peroxisomal lactate shuttle (McClelland et al., 2003). It has long been known that long chain β-oxidation of fatty acids occurs in mammalian peroxisomes (Lazarow and De Duve, 1976), however for β-oxidation to continue, both $FADH_2$ and NADH must be reoxidized. McGroarty and colleagues (McGroarty and Tolbert, 1973) first suggested the presence of LDH in rat liver peroxisomes, however it was not until the study of Baumgart et al., (1996) that LDH was identified in the peroxisomal matrix (Baumgart et al., 1996). McClelland et al., (2003) recently confirmed the findings of Baumgart et al., (1996) identifying the presence of LDH, further, peroxisomal β-oxidation was stimulated by pyruvate, with lactate, generated when pyruvate was added to peroxisomes (Baumgart et al., 1996). MCT1 and MCT2 were identified as facilitating the entry of pyruvate into the peroxisomal matrix and lactate efflux from the organelle, thus forming the basics for a peroxisomal lactate shuttle and explaining how lactate and its efflux can regulate specific cellular and organelle redox balance (Brooks, 2002b)

MCT expression seems to be rapidly modulated to respond to changes in muscle activity. Many studies have demonstrated increases in MCT content following a single exercise bout (Green et al., 2002), or periods of endurance training (Baker et al., 1998, Pilegaard et al., 1999, Dubouchaud et al., 2000). Recent research suggests that MCT increases may occur rapidly following exercise. Zhou et al., (2000) provided evidence that MCT4 mRNA was transiently increased during exercise (Zhou et al., 2000). Further to this, Green et al., (2002) showed an increase in MCT1 (121%) and MCT4 (120%) protein expression taken from skeletal muscle biopsies 2 and 4 days after a 5-6h 60% $_2$peak exercise bout in humans.

Most recently Coles et al., (2004) have shown that 2 hrs (21 m·min^{-1}, 15% grade) exercise in rats increases MCT1 and MCT4 mRNA 2-3 fold, peaking 10 hrs post exercise. These responses however were observed to be tissue specific (different responses found between soleus and EDL muscles) and in some cases transiently upregulated so that protein levels had returned to pre exercise levels 24 h post exercise (Coles et al., 2004). Subsequently these authors suggested that the MCT family of transporters belong to a group of metabolic genes, rapidly activated following exercise (Hildebrandt et al., 2003). These gene products (mRNA) are present in small amounts in cells, however, they have rapid induction times suggesting that small quantities of each are required for metabolic function to be supported (Hildebrandt et al., 2003). It does however remain to be seen whether such rapid induction of MCT's following exercise is repeated in human skeletal muscle. In contrast, denervation (Pilegaard and Juel, 1995) and inactivity (Wilson et al., 1998) leads to a decline in MCT expression.

These discoveries have been important in the recognition of lactate acting as a mobile metabolite, able to move within cellular compartments, adjacent muscle fibres and distributed widely across systemic circulation to inactive tissue and organs. Thus, lactate has the capacity to act as a metabolic signal at the cellular, localised and whole body level, either directly, or through its effects on H^+ or other metabolic regulators. Further, the rapid induction of MCT following repeated muscle contraction means that the mechanisms of lactate transport can quickly adapt to an exercise stimulus.

2.9. Lactate as the cause or consequence of fatigue?

There is a host of research suggesting an association between increased lactate concentration and fatigue during exercise. Initial work by Hill and colleagues (1924) indicated that contraction force of isolated fibres declined at the same time as lactate accumulation increased. Later work by Fabiato and Fabiato (1978) and Allen et al., (1995) demonstrated that the likely mechanism for reduction in force production, by intracellular lactate acidification, was via reduced sensitivity of the sarcoplasmic reticulum Ca^{2+} pump to Ca^{2+} (Fabiato and Fabiato, 1978, Allen et al., 1995).

For some time the release of lactate and hydrogen ions were thought to occur at similar rates inducing lactate acidaemia. However, evidence for dissociation between lactate and hydrogen ion release was demonstrated *in vivo* in humans by Bangsbo et al., (1997). Their study showed that the release of protons can occur, to a large extent, through mechanisms other than diffusion of un-dissociated lactic acid ions during sub-maximal exercise. The non-lactate-

related release of protons was estimated to account for approximately 75% of the total efflux of protons during an exercise bout, leading to the question as to what role lactate may therefore play during muscle contraction (Bangsbo et al., 1997). Posterino and Fryer (2000) further demonstrated *in vitro* that elevated myoplasmic lactate concentrations had negligible effects on voltage-dependent Ca^{2+} handling and muscle contraction at the level of the contractile proteins (Posterino and Fryer, 2000).

General acceptance now is that lactate ions themselves have little effect on muscle contraction (Lindinger, 1995, Posterino and Fryer, 2000), whilst the importance of acidosis in muscle fatigue has also become questioned and may not be such a major factor (Westerblad et al., 2002). Recently, Robergs et al., (2004) reviewed evidence to suggest that there is no biochemical support for lactate production causing all of the intracellular acidosis, with lactate production actually retarding it, perhaps delaying the onset of muscle fatigue, with acidification resulting from other biochemical processes such as ATP breakdown and the earlier stages of glycolysis (Robergs et al., 2004).

Some of the methods employed by Robergs et al., (2004) to illustrate their argument have been questioned by subsequent papers (Boning et al., 2005, Kemp et al., 2006) however the general consensus from a variety of experimental approaches appears to be that lactate has minimal involvement in the onset of fatigue. Instead, recent research suggests an increase of inorganic phosphate (P_i) produced during contraction as the leading contender responsible for initiating muscle fatigue at the level of muscle function (Westerblad et al., 2002). Contemporary explanation of fatigue certainly points

to a combination of effects, as opposed to one mechanism, causing fatigue, certainly in whole organism function. Accordingly, it is probably premature to also accept the P_i hypothesis as the sole cause of fatigue until further research is carried out, particularly *in vivo* (Gladden, 2004) just as care should be taken when dismissing H^+ accumulation from the aetiology of fatigue until our overall understanding of fatigue is improved (Fitts, 1996, Fitts, 2008).

An alternative explanation for lactate release in skeletal muscle could be to have a protective effect on contraction processes (Nielsen et al., 2001). Nielsen and colleagues observed that a reduction in tetanic force of intact isolated muscle fibres caused by elevated potassium (K^+), could be almost completely reversed when incubated in lactate (20 $mmol \cdot l^{-1}$). The substrate concentration used within this experiment led the authors to hypothesise that at high exercise intensities, where intra-muscle lactate is known to range between ~15-25 $mmol \cdot l^{-1}$, lactate acts to increase force, counteracting the force-depressing effects of high extracellular K^+, whilst having no effect on the membrane potential or Ca^{2+} handling of the muscle (Nielsen et al., 2001).

Further research has shown that at a K^+ incubation of 11mM and a temperature of 30°C, a 16% decline in force production of intact rat soleus or EDL can be seen compared to control. At the same K^+ concentration, the previously observed force decrement was restored to control values when temperature (30 to 35°C), lactate (10$mmol \cdot l^{-1}$) and catecholamine concentrations were all elevated, suggesting involvement of each of these factors in force restoration (Pedersen et al., 2003). Further, Karelis et al., (2004) have shown that maximum dynamic and isometric *in situ* force production, of electrically

stimulated rat plantaris muscle, is elevated during intravenous lactate infusion (12 $mmol \cdot l^{-1}$) compared to control. The authors attributed this observation to increased maintenance of M-wave characteristics during electrical stimulation and lactate infusion trials compared to control (Karelis et al., 2004).

Nielsen et al., (2001)'s original lactate protection hypothesis has recently been supported by further work from this group. Pedersen *et al.,* (2004) reported that in the presence of chloride (Cl^-) intracellular acidosis increased the excitability of the T system in depolarized muscle fibres, counteracting fatigue at a critical phase in the excitation-contraction-coupling process. Acidification reduced Cl^- permeability, thereby reducing the stimulus needed to generate a propagating action potential (Pedersen et al., 2004).

This view is not recognised by all. In contrast, Kristensen et al., (2005) questioned whether this phenomenon can be extended to a whole system model during exercise. These authors reported that muscle preparations *in vitro*, were unable to produce a similar amount of force compared to control when incubated in a 20 $mmol \cdot l^{-1}$ Na-lactate, 12 $mmol \cdot l^{-1}$ Na-lactate+8 $mmol \cdot l^{-1}$ lactic acid or a 20 $mmol \cdot l^{-1}$ lactic acid solution and stimulated to fatigue. It was concluded that although lactate helps in regenerating force in passive muscle, this process is not apparent when muscle is exercised (Kristensen et al., 2005). The authors suggest that the depolarizing effect of lactate incubation observed by Nielsen et al., (2001) was not replicated as K^+ depolarisation was less pronounced *in vivo* when muscle was stimulated. This data seems to suggest that the extension of Nielsen et al., (2001) hypothesis to a full system model is difficult due to the number of confounding systems which operate during

exercise *in vivo*. It appears that lactate may delay the onset of fatigue by maintaining the excitability of muscle, and that this situation may happen during extremely intensive exercise. The basis and understanding of this role however still remains poorly understood, whilst currently the methods to transfer isolated muscle research into full system physiology is lacking. Clearly further approaches to investigate this topic are warranted to establish whether Nielsen's hypothesis can be extended to whole muscle function *in vivo*.

2.10. Mechanisms of fatigue in skeletal muscle

The mechanistic basis of exercise induced fatigue has intrigued scientists for well over a century. As mentioned previously, initial investigations on fatigue mechanisms focused on metabolic fuel availability or accumulation of waste products, such as hydrogen or lactate ions in contracting skeletal muscle. Prolonged exercise was thus considered to be limited by reduced muscle glycogen availability and/or reduced circulatory glucose levels, whilst fatigue during intense exercise was typically portrayed as a consequence of phosphocreatine depletion and intracellular acidosis (McKenna and Hargreaves, 2008). The term 'muscle fatigue' has been coined in physiological text as much of the literature during the mid to late 1900's suggested that fatigue was centered to active musculature. However, a growing body of evidence now exists supporting a central component of fatigue and importantly integration between central and peripheral tissue which combines to regulate whole system performance. The following section will summarize current interpretation of the underlying processes regulating fatigue mechanisms in

skeletal muscle and the relevance of this to the contribution of whole system fatigue during exercise.

2.11. Reactive oxygen species and thiol regulation

Compared to the vast bibliography of lactate research in skeletal muscle, the involvement of redox balance in fatigue is a relatively new concept. Over thirty years ago the free radical theory of aging was postulated, identifying the importance of free radicals, and their corresponding antioxidant defence system as central to cellular homeostasis (Harman, 1972). The term 'free radicals' encapsulates a host of cellular products that result due to elevated cellular oxidative stress (Powers and Jackson, 2008). The common free radicals in skeletal muscle, reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in muscle fatigue when cellular concentrations are high (Powers and Jackson, 2008), however there is also some evidence to suggest that ROS generation is an important cellular signal (Scheele et al., 2009).

The generation of reactive oxygen species is an intricate and tightly regulated process. If an atom/molecule contains one or more unpaired electrons and is capable of independent existence, it is referred to as a free radical (Powers and Jackson, 2008). Free radicals can be generated as products of homolytic, heterolytic or redox reactions, producing either charged or uncharged radical species. The primary free radicals generated in cells are superoxide ($O_2^{\cdot -}$) and nitric oxide (NO). Superoxide is generated through either incomplete reduction of oxygen in mitochondria, or as a specific product of enzymatic formation,

whilst NO is produced via Nitric Oxide synthase (NOS) in both mitochondrial and cytosolic compartments (Powers and Jackson, 2008). Importantly for redox balance, superoxide is dismutated by the enzyme superoxide dismutase (SOD) to form hydrogen peroxide (H_2O_2). Hydrogen peroxide has a relatively long half life within the cell and has been suggested to possess cytotoxic properties. H_2O_2 is pivotal in cell metabolism as its catabolism is involved in the generation of free radicals such as the hydroxyl radical which then have potent regulatory effects on muscle contractile machinery (Ferreira and Reid, 2008). The hydroxyl radical ($^{\cdot}OH$) is highly reactive and is the most damaging ROS present in biology (Powers and Jackson, 2008).

Dillard et al., (1978) and subsequently Brady et al., (1979) showed that exercise in rodents and humans respectively was associated with elevated lipid peroxidation in skeletal muscle (Dillard et al., 1978, Brady et al., 1979). For some time mitochondria have been postulated as the principal producers of ROS in skeletal muscle (Boveris and Chance, 1973). However, current opinion is divided as to the importance of mitochondria in this process (Powers and Jackson, 2008) as a number of other sites for ROS production have been identified. The greatest challenge in this area of investigation is that the transient release and volatile nature of ROS production makes their measurement in skeletal muscle very difficult (Ferreira et al., 2008). Accordingly, determining the source of ROS production and metabolism is problematic. What is known is that muscle contraction alters the physiological milieu in skeletal muscle which results in higher levels of ROS production and oxidative stress (Ferreira et al., 2008). Physiological triggers of ROS production are thought to be alterations in temperature, altered O_2 and CO_2 tension and

decreased pH (Powers and Jackson, 2008), however the direct mechanisms by which ROS are produced in skeletal muscle in response to exercise are currently poorly understood (Ferreira et al., 2008).

Importantly, the sites of generation for ROS in skeletal muscle are closely located to important functional sites for muscle metabolism and contraction. The sarcolemma and T tubules contain the enzyme NAD(P)H oxidase which produces ROS in close proximity to ion channels of the t-tubular and the SR terminal cisternae and promotes SR calcium release (Ferreira et al., 2008). At high ROS concentrations, such as following intense muscle contraction, there is clear evidence that ROS can directly affect biochemical processes involved with muscle contraction.

Such ROS mediated modification includes lipid peroxidation, oxidation of mitochondrial and nuclear DNA, heme oxidation, tyrosine nitration, protein carbonylation and thiol oxidation (Ferreira et al., 2008). Of these modifications, the generation of the thiol moiety (-SH) can undergo reversible, covalent reactions with muscle derived oxidants to form disulfide bonds. Thiol oxidation can alter protein function by interfering with biochemical reactions by effecting protein structure and the availability of regulatory sites. A number of important contractile proteins have been shown to undergo reversible thiol-disulfide interactions such as the ryanodine receptor Ca^{2+} release channel (Liu and Pessah, 1994), SR Ca^{2+} ATPase (Xu et al., 1997), troponin (Putkey et al., 1993), tropomyosin (Williams and Swenson, 1982), myosin (Ajtai et al., 1989), actin (Canton et al., 2004) and the Na^{+}/K^{+} -ATPase pump (Ferreira et al., 2008). Support for the key role of thiol generation in muscle fatigue has been provided

by numerous research studies that has shown that the use of the thiol reducer and antioxidant *N*- acetylcysteine (NAC) enhances muscle cysteine and glutathione availability, preserves Na^+/ K^+ pump activity, reduces systemic K^+ concentrations and delays fatigue during prolonged, sub-maximal cycling activity (McKenna et al., 2006, Medved et al., 2003, Medved et al., 2004a, Medved et al., 2004b).

2.12. The importance of calcium for muscle contraction

The importance of calcium fluctuations in skeletal muscle performance have been recognised for a number of decades. Eberstein and Sandow (1963) first demonstrated that caffeine and high extracellular K^+ facilitated SR release of Calcium and attenuated the induction of force (Eberstein and Sandow, 1961). Subsequently, Allen et al., (1989) demonstrated that myoplasmic calcium in isolated muscle fibre preparations increased at the onset of contraction, continued to rise at sub-maximal concentrations but then dropped significantly when fatigue developed (Allen et al., 1989). This functional decline in SR release of calcium is implicit in muscle contraction as restoration of the deficit by caffeine administration increases myoplasmic Ca^{2+} concentrations, offsets fatigue and maintains tetanic force at varying contraction frequencies (Allen et al., 2008). Whilst the decline in myoplasmic calcium is pivotal in skeletal muscle fatigue, the contributing factors which produce this deficit in contracting skeletal muscle are multiple and their relative contributions currently poorly understood (Allen et al., 2008).

Fryer et al., (1995) were the first to postulate that a decline in the amount of stored calcium in the SR may be instrumental in the decline in myoplasmic Ca^{2+} and subsequent fatigue with muscle contraction (Fryer et al., 1995). The key to this mechanism was the observation that accumulation of inorganic phosphate (P_i) in the SR resulted in precipitation of Ca^{2+} and P_i from the SR which ultimately resulted in a reduced amount of Ca^{2+} to be released (Allen et al., 2008). Subsequent research by Westerblad and colleagues (1996) utilizing phosphate injection in isolated skinned rat muscle fibres demonstrated that P_i resulted in a significant decline in SR Ca^{2+} release (Westerblad and Allen, 1996). However, the most conclusive evidence to support the P_i – SR Ca^{2+} hypothesis is the work by Dahlstedt et al., (2000) who utilised a transgenic mouse model deficient in creatine kinase ($CK^{-/-}$). Importantly, these mice show a diminished muscle P_i during fatigue and importantly a slower rate of myoplasmic Ca^{2+} decline during fatigue (Dahlstedt et al., 2000).

2.13. Modification of SR calcium release

As you would expect from such complex contractile machinery, there are a number of mechanisms which can enhance, alter or suppress Ca^{2+} release from the sarcoplasmic reticulum (SR) all of which have consequential effects on skeletal muscle fatigue (Allen et al., 2008). Pivotal to the regulation of SR Ca^{2+} release are the cellular levels of ATP. Traditional interpretation was that ATP levels in skeletal muscle did not decline significantly with exercise due to rapid re-synthesis (Allen et al., 2008). However, Karatzaferi et al., (2001) showed that ATP levels in type IIX fibres declined by ~80% following cycling exercise to exhaustion in humans (Karatzaferi et al., 2001a, Karatzaferi et al., 2001b).

Further, the authors suggest that this decline is compartmentalised in muscle fibres, so that in practice, where ATP levels are in highest demand for contraction, such as in close proximity to myosin heads, SR Ca^{2+} pumps or Na^{+}-K^{+} pumps the decline may have been more substantial. The importance of this decline is clear when you consider functionally what ATP decline means for cellular regulation (Allen et al., 2008). ATP in a rested state has high affinity for magnesium (Mg^{2+}). During contraction, ATP is metabolized and there is an increased content of ADP, AMP and IMP. In contrast to ATP; ADP, AMP and IMP has much lower affinity for Mg^{2+} and so this results in an increased amount of free Mg^{2+} in the cell. The relevance of this alteration in free Mg^{2+} is that Mg^{2+} has been shown to inhibit the Ca^{2+} release channel in the SR by approximately 40% when Mg^{2+} increased ~2 fold (Laver et al., 1997). Interestingly, there also appears to be a close relationship between Mg^{2+} concentrations and the inhibitory effect of P_I on SR Ca^{2+} release (Duke and Steele, 2001) further implicating the importance of free Mg^{2+} in muscle contraction (Allen et al., 2008).

ATP levels can also directly affect Ca^{2+} release from the SR, either by direct binding to the Ca^{2+} release channel (Dutka and Lamb, 2004a, Dutka and Lamb, 2004b), or again via ADP and AMP generation which antagonizes the effect of the remaining ATP on Ca^{2+} release channel function (Dutka and Lamb, 2004a, Dutka and Lamb, 2004b). Functionally, the suppression of Ca^{2+} release will inhibit muscle contraction due to reduction in cross-bridge cycling and SR Ca^{2+} uptake, the two main processes involved in ATP hydrolysis. This results in reduced power output, which during whole body exercise would result in performance decline and termination of exercise (Allen et al., 2008). Further,

the ability of ATP levels to regulate muscle contraction is logical as it provides a protective mechanism during intense muscle contraction. Shut down of the system via reduced SR Ca^{2+} release would guard against complete ATP depletion. This then stops excessive cellular damage and the development of rigor (Allen et al, 2008).

2.14. The central component of muscle fatigue

Whilst localised muscle fatigue clearly plays a pivotal role in the aetiology of exercise induced fatigue, there is a central component of fatigue which warrants discussion (Gandevia, 2001). Probably the biggest limitation to this area of research in humans is the difficulty in quantifying central fatigue during exercise in humans (Gandevia, 2001). Central fatigue can be broadly defined as a progressive exercise-induced failure to voluntarily activate skeletal muscle (Taylor and Gandevia, 2008). Evidence of this phenomenon can be demonstrated via electrical stimulation *in vivo* pre and post exhaustive exercise. Herbert and Gandevia (2001) demonstrated that superimposing a muscle contraction in the quadriceps muscle via electrical stimulation post exercise increased the force production during an MVC (Herbert et al., 2002). This was suggestive of insufficient voluntary excitatory input to the muscle signifying central fatigue, and indicating that central processes proximal to the site of motor axon stimulation are contributing to a loss of force.

In addition, Gandevia et al., (1996) have suggested that there is a supraspinal component of central fatigue. Gandevia et al., (1996) used transcranial magnetic stimulation (TMS) of the motor cortex during maximal elbow flexion

movement to demonstrate that motor cortical output is not maximal at the moment of contraction (Gandevia et al., 1996). The authors observed a superimposed twitch with the TMS stimulation, despite maximal effort of contraction. This suggested that motor cortical output is not optimal, and that the increase in the superimposed twitch elicited by cortical stimulation during exercise is the marker of supraspinal fatigue (Taylor and Gandevia, 2008).

There is clear evidence from a number of independent research groups that a slowing of motor unit firing rates occurs during sustained and repeated maximal efforts (Bigland and Lippold, 1954, Marsden et al., 1971) and that this phenomenon occurs in both young and elderly individuals (Rubenstein and Kamen, 2005). Whilst the precise mechanisms for this decline remain uncharacterised, Taylor and Gandevia (2008) categorise three clear processes which will affect motor unit firing rate either individually, or collectively, during exercise. Firstly, firing rate will decline due to a decrease in excitatory input; secondly an increase in inhibitory input will suppress motor neuron firing capacity and finally, a decrease in the responsiveness of the motorneurons through a change in their intrinsic properties.

Repetitive sub-maximal activation has been suggested to decrease the responsiveness of synaptic input which ultimately leads to weak voluntary contractions (Nordstrom et al., 2007). In combination with the reduction in synaptic input responsiveness is a change to the input received by the motorneuron (Taylor and Gandevia, 2008). The inputs that are most likely to change include reflex inputs from the motor afferents, recurrent inhibition and descending drive (Taylor and Gandevia, 2008). Firing of Golgi tendon organs is

also likely to decrease with the fall in muscle force during fatiguing maximal contractions (Zytnicki et al., 1990). In particular, small diameter (groups III and IV) muscle afferents are variously sensitive to mechanical and chemical stimuli so that some increase firing with the accumulation of metabolites in the fatigued muscle (Zytnicki et al., 1990).

Unfortunately, there is limited information regarding central fatigue during sub-maximal, whole body exercise, with the majority of studies using maximal voluntary contractions in large muscle groups such as the quadriceps, or in small muscle groups such as the wrist flexors (Gandevia, 2001). What sub-maximal studies have shown is that individual perceived effort of exertion is important for maintaining contractile force, and so appears to indicate the onset of central fatigue. During sub-maximal MVC's (15% of maximal) subjects reported a mild initial effort, which increased to a very large effort after ~40 minutes of exercise (Sogaard et al., 2006) Of interest was the fact that during this increase in perceived pain, EMG RMS amplitude in the active muscles only increased to 35% of maximal levels, indicating clear discordance between the perception of muscle fatigue and actual mechanical fatigue. Smith et al., (2007) also identify a similar phenomenon to occur during a prolonged 5% MVC over a 70 minute period (Smith and Newham, 2007).

Peripheral or localised fatigue is characterised by metabolic change in specific skeletal muscle or muscle groups, whether it be a reduction in pH, or an increased accumulation of a compound such as P_i. The classical theory of exercise induced fatigue proposes that exercise is limited only after oxygen

delivery to the exercising skeletal muscle becomes inadequate, inducing anaerobiosis (Mitchell and Blomqvist, 1971, Bassett and Howley, 2000).

Noakes and colleagues have suggested an alternative hypothesis, implicating a 'central governor' (CNS), which regulates the mass of skeletal muscle recruited during exercise through motoneurone pool recruitment, a consequence of which would be to protect the heart from ischaemia during maximal exercise (Noakes, 2007, Noakes et al., 2001, St Clair Gibson et al., 2003, St Clair Gibson et al., 2006). This model predicts that the ultimate control of exercise performance resides in the brain's ability to vary the work rate and metabolic demand by altering the number of skeletal muscle motor units recruited during exercise (Noakes et al., 2001). Some attempts have been made by this group to address physiological parameters in peripheral tissue which may act as the signal to the CNS to regulate exercise intensity (Rauch et al., 2005), however this mechanism still remains unclear.

So could lactate have a role as a peripheral signal to the CNS during exercise? We now know that lactate is a mobile metabolite capable of cell and intracellular shuttling, with the circulation able to shift this metabolite to a number of facultative sites for oxidation or recycling. There is also mounting evidence in support of lactate utilisation in the brain (Ide et al., 2000, Pellerin and Magistretti, 2003a, Pellerin and Magistretti, 2003b) via the astrocyte-neuron lactate shuttle, a system clearly capable of affecting substrate delivery and neurone function (Pellerin et al., 1998a, Pellerin et al., 1998b). So could lactate be one of the peripheral exercise signals that might be incorporated into Noakes' model (Noakes, 2007)? Certainly, lactate's production characteristics

allow it to perform such a role. It is elevated during exercise and reaches maximal levels at or just following the termination of exercise. Further, shuttling mechanisms would allow for an influence of lactate, centrally and peripherally, again fulfilling roles as part of the 'central governor' hypothesis. It will be of interest as to whether the peripheral signal for the central governor is identified in future research and whether lactate has a role to play in this scenario.

2.15. ROS act as contraction derived signals

The observation that exercise resulted in transient elevations in oxidative stress has interested researchers for almost three decades, however, contemporary research still is unable to define the exact mechanism for this phenomena (Powers and Jackson, 2009). There is strong evidence to suggest that muscle contraction results in an increase in the generation of reactive oxygen (ROS) and nitrogen species (RNS) which have been directly implicated in the onset of fatigue during sub-maximal and maximal muscle contractions (Ferreira and Read, 2008). In addition, there is a growing body of evidence to suggest that the generation of these reactive species may operate as a signaling mechanism in skeletal muscle, and as such play a key role in co-ordinating muscle adaptation to exercise (Scheele *et al*., 2009). This suggestion is also somewhat paradoxical as oxidative stress is a recognized consequence of chronic inflammatory states such as type 2 diabetes and insulin resistance (Scheele *et al*., 2009).

Recently, however, St Pierre and coworkers (2006) demonstrated that PGC-1α knockout mice express reduced levels of the antioxidant enzymes glutathione

peroxidase-1 and superoxide dismutase-2 (SOD2), and accordingly show increased neurodegenerative disease as a consequence of oxidative stress damage. When the same group virally over-expressed PGC-1α in isolated neural cells from these mice they observed a protective effect of PGC-1α on ROS mediated damage, suggesting a direct mechanism for PGC-1α to regulate antioxidant defense systems (St-Pierre et al., 2006). This would suggest two important conclusions, firstly that reduced PGC-1α causes reduced mitochondria, reducing cellular oxidative capacity and ROS generation, suggesting specific crosstalk between PGC-1α and ROS. Secondly, ROS might regulate antioxidant defense gene transcription via ROS sensitive transcription factors suggesting an important integrative role for ROS in coordinating cellular antioxidant cell systems.

Recently, Irrcher et al., (2008) demonstrated that hydrogen peroxide (H_2O_2) treatment of C2C12 myotubes increased a number of antioxidant enzymes and importantly a 3-fold increase in PGC-1α mRNA expression. The authors demonstrated that H_2O_2 increased PGC-1α activity via an E-box repeat in the proximal PGC-1α promoter. Interestingly, Irrcher et al., (2008) also demonstrated that AICAR treatment, which increased AMPK activity also increased PGC-1α promoter activity via the E-box repeat. Both treatments appeared to mediate this response via activation of the transcription factor upstream factor 1 (USF1) which was shown to associate with the E-box domain and increase PGC-1α activity via chromatin immunoprecipitation (ChIP) analysis (Irrcher et al., 2009).

2.16. Lactate as a signalling molecule

It appears that we still do not fully understand all of the roles for lactate *in vivo*. Whilst much of the data presented so far has been gleaned from isolated muscle, or cell culture, understanding how these observations transfer to the whole organism is perhaps the next important question to be addressed.

Suggestion of a role for lactate as a metabolic signal at the whole organism level has been postulated by Brooks (Brooks, 2002b), who proposed that lactate may operate as a pseudo-hormone. Within this model, blood glucose and glycogen reserves in diverse tissues are regulated to provide lactate, which may then be used within the cells where it is made, or transported through the interstitium and vasculature to adjacent or anatomically distributed cells for utilization. In this role lactate becomes a quantitatively important oxidizable substrate and gluconeogenic precursor, as well as a means by which metabolism in diverse tissues may be co-ordinated. Lactate has the ability to regulate cellular redox state, via exchange and conversion into its more readily oxidized analogue, pyruvate, and effects on NAD^+/NADH ratios. Lactate is released into the systemic circulation and taken up by distal tissues and organs; where it also affects the redox state in those cells (Brooks, 2002b).

Further evidence for lactate acting as something more than a metabolite or metabolic by-product comes from wound repair research where lactate appears to induce a biochemical ‘perception’ effect (Trabold et al., 2003). It had been suggested that the elevated acidosis associated with wound regeneration was a result of localised hypoxia. However, Trabold et al., (2003) provided evidence

that lactate may act as a stimulus similar to hypoxia without any compromise to O_2 levels. Green and Goldberg (1964) demonstrated that collagen synthesis rose ~2 fold in lactate incubated (15 $mmol \cdot l^{-1}$) fibroblasts, whilst Constant et al., (2000) showed that increased lactate was capable of upregulating Vascular Endothelial Growth Factor (VEGF) in similar proportions (Green and Goldberg, 1964, Constant et al., 2000). To examine this apparent relationship, Trabold et al., (2003) elevated extracellular lactate in the wounds of male Sprague-Dawley rats by implanting purified solid–state, hydrolysable polyglycolide. This substance raised localised lactate to a maintained 2-3 $mmol \cdot l^{-1}$. Elevating lactate resulted in elevations in VEGF and a 50% increase in collagen deposition over a 3-week period. This data suggests that lactate is capable of inducing responses characteristic of O_2 lack, operating to instigate a pseudo-hypoxic (as far as concentration of lactate is concerned) environment. In combination with this action, the continued presence of molecular oxygen (as the tissue was not hypoxic) allows endothelial cells and fibroblasts to promote increased collagen deposition and neovascularization.

Hashimoto et al., (2007) were the first to demonstrate that lactate may act as a metabolic signal when they identified a lactate sensitive transcription factor network in L6 myotubes. The lactate signalling cascade appears to be mediated via reactive oxygen sensitive transcription factors, as acute lactate treatment increased H_2O_2 concentrations. Specifically, incubation of myotubes with 20$mmol \cdot l^{-1}$ lactate increased MCT1 expression 1h post treatment suggesting that lactate is capable of regulating its own transporter. Secondly, lactate increased the binding of NF-κB and NF-E2 to the MCT1 promoter, suggesting that this ROS sensitive mechanism was in part responsible for the upregulation

of MCT1. Further, 20mmol·l^{-1} lactate increased the expression of PGC-1α whilst also targeted the increase of 673 genes involved in substrate transport and cellular metabolism (Figure 2.4)(Hashimoto et al., 2007).

Figure 2.4. The lactate transcriptional network in L6 myotubes. Exercise activates a number of cellular signalling pathways in skeletal muscle including lactate release. Lactate, via ROS phosphorylates Calcium dependant calmodulin kinase 2 or protein kinase C which increases the activity of the transcriptional co-activator PGC1α which in turn can bind to transcription factors and activate promoter regions of genes involved in metabolic adaptation (Hashimoto et al., 2007).

The possibility that lactate acts as a metabolic signal is important to take research further. However the described work by Trabold et al., (2003), Brooks (2002a) and Hashimoto et al., (2007) is gleaned from work *in vitro*. Can a working model of lactate signalling be extended to systemic and localised exercise function? Firstly, lactate could, potentially, influence local and central

blood flow during exercise. Hypoxia is known to stimulate systemic vasodilation via a host of neural, hormonal and local factors (Skinner and Marshall, 1996).

Fattor et al., (2005) have recently used the lactate clamp method to demonstrate an auto-regulatory loop in sympathetic drive that is governed by lactate release (Fattor et al., 2005). Circulatory norepinephrine was reduced during exercise at 65% $\dot{V}O_2$peak when lactate was maintained at 4mmol·l^{-1} compared to control (2.115 ± 166 pg/ml to 930 ± 174 pg/ml respectively) with epinephrine concentrations displaying a similar trend (EX; 262 ± 37 pg/ml to LC; 113 ± 23 pg/ml). This lends evidence to the possibility of modulatory control of catecholamines by lactate. The infusion of lactate had no effects on other glucoregulatory hormones (i.e. insulin and glucagon) or cortisol. The authors suggest that the lactate anion was sensed by either the ventromedial hypothalamus (VMH) or elsewhere via neuronal metabolism signalling abundant fuel supply; however this theory remains to be tested (Fattor et al., 2005). Therefore, the release of lactate into the circulation at the onset of exercise could promote vasodilation allowing oxygenated blood to reach active muscle, acting in an additive or modulatory manner to the demands of tissues during exercise.

2.17. A role for lactate in fuel selection?

In many vertebrate species so far examined, fuel selection has been shown to correlate closely with exercise intensity (Roberts et al., 1996, Bergman and Brooks, 1999, Conley et al., 2001). At rest and moderate exercise intensities fat oxidation is the predominant source of ATP production. As exercise intensity

rises, a proportional increase in carbohydrate oxidation occurs with lactate production following this trend (Figure 2.5).

Figure 2.5. Contribution of carbohydrate (CHO) and free fatty acids (FAT) to total substrate oxidation during exercise before (UT) and after (T) training (Bergman and Brooks, 1999b).

This co-ordinated control was first identified and named the Glucose Fatty–acid cycle by Randle and colleagues (1964). In short, elevated glucose concentrations stimulate the secretion of insulin, which suppresses non-esterified fatty acid (NEFA) release from adipose tissue, altering fuel use and supply, leading to the preferential use of carbohydrate. In the reverse situation, when plasma NEFA concentrations are elevated (e.g. during starvation, exercise or low insulin levels), fatty acids are predominantly released and oxidised and glucose levels observed to be low (Randle et al., 1964, Newsholme and Randle, 1964, Garland et al., 1964).

Brooks and Mercier (1994) recognised that a clear crossing point where fuel utilisation came from fat and CHO equally was observable in fuel selection. The 'crossover concept' suggests that the proportion of substrate utilization in an individual at any point in time depends on a trade-off between exercise intensity induced responses (which increase CHO utilization) and endurance training-induced responses (which promote lipid mobilisation and oxidation). The crossover point may be taken as the power output at which energy from CHO-derived fuels predominates over that from lipids, with increases in power eliciting further increments in CHO utilization and decrements in lipid oxidation (Brooks and Mercier, 1994).

The exercise intensity at which a transitional shift in substrate supply might occur was originally examined in dogs and goats by Roberts et al., (1996) through calculated rates of fat and carbohydrate oxidation from respiratory exchange ratio (RER) data. Maximal fat oxidation rates were observed at 40% of maximal exercise intensity in both species, with fat oxidation shown to provide around 77% of total energy requirements. Bergman and Brooks (1999) studied this in humans, and found the highest lipid oxidation rate in the fed state at 40% VO_2peak (Figure 2.6). Taken together, the data provided by Roberts et al., (1996) and Bergman and Brooks (1999) would suggest that humans and other mammals, regardless of differences in aerobic capacities, genotype and training adaptation demonstrate similar substrate utilization patterns when relative exercise intensity is considered (Bergman and Brooks, 1999). Van Loon et al., (2001) utilised a continuous infusion of [U-^{13}C] palmitate and [6,6-$^{2}H_2$] glucose to provide direct measures of whole-body fat oxidation and glucose turnover, which were increased from rest at approximately 8 kJ min^{-1} up to a

maximum rate of 32 ± 2 kJ min^{-1} at 55% maximal workload (W_{max}) or approximately 60 – 75% maximal oxygen consumption. As exercise intensity increased to 75% $W_{max,}$ fat oxidation declined by 34% to 19 ± 2 kJ min^{-1}. FFA concentrations and blood flow were maintained at the highest exercise intensity suggesting ample FFA arterial availability (van Loon et al., 2001) (Figure 2.6).

Figure 2.6. Substrate utilization at progressive exercise intensities (Van Loon et al., 2001)

Three possibilities have been suggested to explain the decline in FFA acid oxidation in the face of sufficient supply. Firstly, gradual depletion or limited turnover of the cytosolic free carnitine pool could alter long chain fatty acid (LCFA) transport across the mitochondrial membrane (Harris and Foster, 1990). Secondly, reduced transport of free fatty acids by escalating cellular or systemic acidosis (FFA) may limit FFA uptake, due to down regulation of the fatty acid transporter, carnitine palmitoyl-transferase 1 (CPT1) (Sidossis et al., 1997). Finally, changes in glucose flux and energy expenditure may regulate the

amount of available malonyl-CoA, an allosteric inhibitor of CPT1, which has been shown to regulate fat oxidation (Ruderman and Dean, 1998, Roepstorff et al., 2006). To date, the exact mechanism regulating the relative contribution of carbohydrate and fat to energy provision during exercise still remains unknown. The most recent examination of fuel balance during exercise was conducted by Roepstorff et al., (2006) who utilised high or low CHO diets to influence glycogen stores and substrate utilisation during 60 min bicycle exercise at 65% VO_2peak in 8 healthy male subjects. The authors observed a decline in muscle malonyl-CoA concentrations from rest to moderate intensity exercise, however there was no change observed when fat oxidation rates were altered by the pre exercise meal. Thus the authors concluded that malonyl-CoA may have a role in increasing absolute levels of fat oxidation; however it would not appear to play a major part in fine tuning the shifts in CHO and fat oxidation during the rest to exercise transition or during sustained exercise. In contrast, the availability of free carnitine to CPT1 appears to participate in regulating fat oxidation during exercise, as muscle carnitine and fat oxidation rates were both lower during exercise with high compared to low glycogen conditions (Roepstorff et al., 2006).

So is there potential for lactate to play a role in effecting this transition? Previous research has shown that, in isolated mitochondria, a reduction in pH decreases the activity of CPT1 by increasing the K_m of CPT1 for carnitine (Mills et al., 1984). Starritt et al., (2000) demonstrated that a decrease in pH from 7.0 to 6.8 reduces CPT1 activity by 40% *in vitro*, thereby offering a potential mechanism for extracellular acidosis to inhibit fat oxidation by reducing supply to the mitochondria, or reducing the rate of fat oxidation at lower exercise

intensities where a fall in pH of approximately 0.1-0.3 units is common (Starritt et al., 2000).

There is a host of research suggesting a direct effect of lactate on inhibition of lipolysis and increased re-esterification of FFA (Jeukendrup, 2002). Whilst it seems that this evidence supports a role for acidification in reducing fatty acid metabolism, it is not clear whether this can be attributed to an increase in H^+, lactate alone or a combination of each. Most recently, Corbett et al., (2004) have shown that as plasma lactate increases at progressive exercise intensities, so NEFA levels decline.

If we put this data into a physiological context, it is known that the lactate threshold (a sustained increased in systemic lactate from resting levels) during exercise occurs in most subjects at 60 – 75% $\dot{V}O_2$max with the accumulation of circulatory lactate known to increase non-uniformly beyond this exercise transition (Corbett et al., 2009). This relationship could, of course, be chance, with lactate increase solely due to increased carbohydrate oxidation or glycolytic flux. However, if we examine the increase in lactate in the context of a signalling hypothesis, lactate's role could be perceived as something very different.

It is known that ample tissue oxygenation is available in skeletal muscle at intensities approximately 60%–75% $\dot{V}O_2$max, allowing oxidative phosphorylation to proceed (Richardson et al., 1998) so lactate is not released due to tissue hypoxia. Similarly, lactate will be maintained at a steady state beyond the lactate threshold, up to a maximal lactate steady state, indicating

that lactate clearance capacity is not exceeded at these conditions (Billat et al., 2003). Could it be that lactate is released to signal a progressive switch in fuel utilisation from fat to carbohydrate, reducing FFA substrate availability for the CPT complex, whilst also acting perhaps in combination with H^+ accumulation to reduce pH, subsequently down regulating CPT1 facilitated FFA transport? This model may provide an efficient way of regulating fuel supply as lactate is produced, signals to its targets and is then re-used as a fuel, allowing continuation of glycolysis and oxidative phosphorylation.

As previously discussed, lactate is preferentially utilised, compared with glucose and pyruvate, in cardiac muscle (Laughlin et al., 1993). Further, Chatham et al., (2001) have reported a similar selectivity for ^{13}C lactate to be preferentially oxidised ahead of ^{13}C glucose, again in cardiac muscle preparations. Miller et al., (2002) extended this observation when they reported that infused lactate was oxidised in preference to glucose at rest and during whole body exercise in humans. The authors concluded that lactate, provided by intravenous infusion, acted in a glucose sparing role, allowing glucose and glycogen stores to be maintained, to be utilized later in periods of increased exercise stress (Miller et al., 2002).

Artificially elevating lactate concentrations, such as the lactate clamp method utilised by Miller et al., (2002) allows for the investigation of lactate's role on a variety of processes, however it does provide a non-physiological situation, as lactate is added independently of glucose usage. The elevated lactate concentrations could therefore serve to stunt glycolysis, as opposed to sparing glucose concentrations. Infused lactate, if the intracellular lactate shuttle is

indeed correct, will by-pass glycolysis, becoming readily accepted into the mitochondria where it is converted to pyruvate via mLDH. Therefore lactate synthesis in the cytosol would be reduced, and an increase in H^+ would follow, since lactate production from pyruvate normally accepts a H^+ from NADH. This increased acidification could suppress glycolysis by inhibiting PFK activity whilst affecting the redox state of the cell. Glucose and glycogen would then be spared by lactate oxidation, however this process cannot occur during regular exercise as without the infusion, the only source of lactate production would be as a consequence of glycolysis.

There is good evidence to support a lactate signalling hypothesis during exercise (Philp et al., 2005)(Figure 2.7). In this scenario, lactate becomes mobilised at the onset of exercise (rate depending on mode and intensity of exercise). During this exercise transition there is approximately a 2 fold elevation in circulating lactate (~1-2 min increasing lactate to 1-2mmol$\cdot l^{-1}$) and this concentration is greater still in muscle and interstitium. Lactate has a modulatory effect on vasodilation and catecholamine release stimulating fat and carbohydrate oxidation. Lactate is then shuttled from its site of production in the cytosol to adjacent muscle fibres where it is reconverted to pyruvate and enters the TCA cycle for oxidative phosphorylation or mobilised into the circulation where it is reconverted to glucose by the liver, therefore providing an efficient signal-to-fuel process as lactate is recycled during gluconeogenesis or oxidised. In turn lactate also promotes vasodilation of active musculature and stimulates ventilatory drive (Gargaglioni et al., 2003, Hardarson et al., 1998) (Figure 2.7).

Figure 2.7. Mechanisms by which lactate may influence cell and systemic function during exercise (Philp et al., 2005)

As exercise progresses into the moderate to high exercise zone (65-85% $\dot{V}O_2$max; lactate 2-10mmol·l^{-1}) lactate production exceeds the removal capacity of the MCT transport system. Lactate and H^+ ions influence CPT1 function thereby reducing fat oxidation, prompting a shift towards carbohydrate oxidation becoming the predominant fuel utilised. Availability of O_2 is still adequate for oxidative phosphorylation; however, the presence of lactate simulates conditions that may be recognised as hypoxic in nature, influencing angiogenesis, oxidative defence mechanisms and collagen synthesis all serving to improve muscle function. Lactate has also been previously suggested to act as a scavenger for free radicals released into the circulation (Groussard et al., 2000) and could potentially operate in this manner as exercise intensity increases (Figure 2.7).

As exercise progresses towards exhaustion, whole body lactate levels continue to rise (detectable as 8-20mmol·l^{-1} in blood and higher in muscle). ATP provision in active muscle is approaching its maximal capacity and there is a gradual decline in cellular and systemic pH. Elevated lactate helps reduce glucose usage and glycogenolysis minimising depletion of these stores as escalating acidosis reduces PFK function. In this role, lactate is filling a dual purpose. Firstly its release is indicating stress placed upon active muscle, whilst secondly high concentrations of intracellular lactate could potentially be acting in a protective manner. Acting as a peripheral signal, lactate could therefore provide a mechanism by which the CNS detects localised, at the level of muscle or muscle group, exercise stress and causes exercise to terminate (Noakes et al., 2001).

2.18. Thesis aims

As the transition point between the high and severe exercise intensity zones, the intensity at maximal lactate steady state (MLSS) appears to represent a fundamentally important point in the regulation of endurance exercise. There is, however, a surprisingly small amount of research examining the fundamental basis of this intensity, and the mechanisms regulating substrate balance at the MLSS, and at work intensities around this marker. Specifically this thesis will aim to address the following research questions.

1. Does the currently accepted method of classifying the MLSS provide an intensity that is robust to repetitive exercise bouts and circadian variation?

2. How does the MLSS respond to endurance training and specifically can the intensity at MLSS be used as a training stimulus to promote training adaptation?

3. Is lactate accumulation the only physiological mechanism potentially responsible for fatigue during exercise to exhaustion at and above the MLSS?

4. What is the mechanism for increased lactate production and exercise termination during exercise to exhaustion at and above the MLSS?

CHAPTER III - GENERAL METHODS

The following chapter details the methods used routinely throughout this thesis. Specific techniques used in individual experimental chapters are reported in full in the appropriate chapter method section.

3.1. Location

All experimental data collection and analyses were performed in the British Association of Sport and Exercise Science (BASES) accredited exercise physiology laboratories, University of Brighton, Eastbourne.

3.2. Environment

All experimentation took place in air conditioned laboratories maintaining ambient temperatures at 20°C. Air pressure and relative humidity ranged from 740 to 770 mmHg and 33 to 55% during the experimentation periods.

3.3. Health and Safety

Prior to and following each experiment all equipment was cleaned thoroughly using appropriate materials. Disposable latex gloves were worn during the obtaining and handling of biological samples with generated waste disposed of using appropriate storage facilities and subsequent incineration. During each trial, electric fans were available on request to cool the participants while water was provided *ad libutum* when required.

3.4. Ethical Clearance and Participant Recruitment Criteria

Ethical approval for the experimental work described in this thesis was granted by the East Sussex Local Research Ethics Committee (LREC) and the University of Brighton Ethics Committee. All experimental procedures were conducted in accordance with the World Medical Associations revised declaration of Helsinki for medical research involving humans (World Medical Association, 2008).

Participants were recruited via local advertisement. Specific recruitment criteria were that each subject was physically active, injury free at the time of commencing the study and did not have a respiratory, cardiac or metabolic condition that would inhibit their exercise performance. Volunteers were recruited from local student and general public populations in Eastbourne via mail, poster and email advertisements.

3.5. Informed consent

On expressing interest in the study, participants received an information sheet detailing the experimental procedures, individual requirements during the study and any potential risks or discomforts involved in the experimental testing. Following this, written informed consent and a completed medical questionnaire were obtained from each volunteer at the onset of the investigation and updated accordingly during the course of the specific study. All data collected was stored in a locked filling cabinet or password protected computer. Participants were kept anonymous during any dissemination of results.

3.6. Standardisation of visits

All experimental testing carried out within this thesis has undertaken the following standardisation of visits to the lab:

- Habituation visit where all exercise equipment and collection techniques are described to the participant.
- An incremental test to determine the individual work intensity at the Lactate Threshold (LT) and maximal oxygen consumption ($\dot{V}O_2$max).
- Between 3 and 5 constant load exercise tests to determine individual work intensity at the Maximal Lactate Steady State (MLSS).

Prior to each visit, participants were asked to refrain from vigorous exercise and alcohol in the 48h preceding their visit and to abstain from caffeine and any medication in the 24h preceding their laboratory session. Each subject was asked to record a diet diary for the 48h preceding exercise testing and repeat this diet prior to each subsequent visit. During the latter experiments of this thesis participants were given a set meal prior to exercise trials. The specific calorie yield of each meal is detailed in the appropriate chapter.

3.7. Anthropometric Data

Prior to each study, participants' height, body mass and percentage body fat was recorded. Height was measured to within 0.1 cm using a Harpenden stadiometer with participants standing without shoes and the head in the Frankfurt plane. Body mass was measured to an accuracy of 0.1 kg with

participants standing barefoot whilst wearing minimal clothing. Percentage body fat was estimated by the four-site skinfold equation of Durnin and Womersley (Durnin and Womersley, 1974).

3.8. Electronically braked cycle ergometer

All cycle tests were conducted on an electronically braked cycle ergometer (Jaeger Ergoline ergometric E800, Netherlands). Seat, handlebar height and angle were kept constant for each individual participant. Participants remained seated throughout each exercise trial and maintained a pedal rate of 75 ± 5 rpm.

3.9. Motorised treadmill ergometer

All running tests were performed on a motorised treadmill (Woodway ELG2, Cardiokinetics, Salford, UK). Treadmill belt speed was regularly checked and calibrated when required. The treadmill gradient was maintained at 1% as previously recommended to allow comparison with the demands of outdoor running (Jones and Doust, 1996).

3.10. Classifying the Lactate Threshold and Maximal Oxygen consumption

Participants completed an incremental exercise test on an electronically braked cycle ergometer (Jaeger, Wurzburg, Germany) to determine LT and $\dot{V}O_2$max. Seat and handlebar height and angle were kept constant for individual

participants and a five minute warm up of pedalling at 60W preceded each test. At the initiation of each trial, pedal rate was controlled at 75 ± 5 $rev \cdot min^{-1}$ and kept constant throughout testing. The initial starting power output (W) for each trial was 100W, with participants performing four minute stages with gas exchange measured continuously (Figure 3.1). During the last ten seconds of each stage a blood fingertip sample was obtained for the determination of whole blood lactate and glucose. Heart rate (Polar Electro Oy, Kempele, Finland) and RPE were also recorded.

Figure 3.1. Representation of an incremental test protocol to determine the lactate threshold (LT). Triangles denote the plasma lactate response to exercise of increasing intensity.

The protocol continued with 20W increments until individual LT was reached, defined as the first and sustained increase in lactate from baseline (Jones and Carter, 2000). Following this point, the increment duration changed to one minute stages until volitional exhaustion was reached. A final fingertip sample was obtained 3 mins post exercise. The blood lactate response was plotted against individual power output and $\dot{V}O_2$ values. LT was determined by two

independent reviewers, whilst the highest minute average of the breath-by-breath $\dot{V}O_2$ data was taken to represent $\dot{V}O_2$max.

3.11. Classification of the Maximal Lactate Steady State

Following the initial test protocol, participants revisited the lab on three occasions for the determination of MLSS. Initial testing took place at approx 50W or 1km·h^{-1} above LT. Following a five-minute warm up at 50W, participants completed a 30-minute constant load exercise protocol. Pulmonary gas exchange and heart rate were recorded continuously throughout whilst a fingertip blood sample and RPE was recorded at five minute intervals. MLSS was characterised as the power output corresponding to an increase in blood lactate of no more than 1mM during minutes 10-30 of the protocol (Beneke and von Duvillard, 1996) (Figure 3.2).

Figure 3.2. Example of typical lactate response curves to incremental and constant load exercise. Note, the MLSS is defined as the highest power output at which a steady state in lactate is achieved in minutes 10-30 (yellow triangles).

3.12. Collection of capillarised blood

Briefly, the fingertip site was cleaned using an alcohol swab (Alcowipes, Seton Health Group PLC, Oldham, UK), dried with a tissue, before a small puncture was administered (approximately 2mm in depth) by a disposable safety lancet (Softclix Pro, Accu-Check, Manheim, Germany). The initial droplet of blood was wiped clear before approximately 50-100 µl of blood was collected into capillary tubing containing a lithium heparin solution (Hawksley and Sons, Lancing, UK). Samples were immediately analysed for lactate and glucose using the glucose oxidase reaction on an automated analyser (YSI 1500, Yellow Springs, CA, USA), previously calibrated against a standard of known concentration.

3.13. Collection of venous blood

An 18 gauge cannula (Biovalve, Ecouen, France) was inserted into an anticubital vein by a NHS qualified phlebotomist allowing frequent sampling of venous blood. Venous blood was drawn by a 10ml syringe (BD Medical, Singapore) and dispensed into 5ml tubes containing either lithium heparin, EDTA or no anticoagulants depending on the relevant metabolite analysis required. Each cannula was maintained during experimental trials by a constant, slow drip containing 0.9% NaCl, Baxter Healthcare Ltd, Thetford, England).

3.14. Collection of arterialised blood

At rest and during exercise, as blood passes through skeletal muscle, there is known metabolite exchange between the circulation and tissue bed. This exchange is evident in specific arterial-venous difference, with venous blood values almost exclusively lower than arterial values. Therefore in order to accurately measure whole body metabolite and hormonal levels before organ and skeletal muscle uptake, arterial values must be attained. Arterial sampling is a potentially hazardous procedure providing a certain amount of discomfort to an individual.

The collection or arterialised blood offers a practical alternative to arterial measurements. Previous research has shown that when the surface area of the hand is heated to temperatures exceeding 37°C the capillaries of the hand dilate to increase blood flow to the surface as a cooling mechanism. In order to suitably heat the hand, a surrounding air temperature of 60°C will result in venous blood, as collected in a retrograde manner, will yield ~98% arterialised blood (Clutter et al., 1980). To generate an environment allowing this process to take place, each participant placed their hand in a thermoregulated box (Figure 3.1) for the duration of each experimental trial. A dorsal hand vein was cannulated as previously described, with the cannula maintained using a continuous saline infusion.

3.15. Centrifugation and Storage Procedures

Blood samples, once collected, were placed in 5ml tubes containing an anticoagulant. Each sample was slowly inverted a number of times to ensure the sample was sufficiently coated before being stored at 4°C. Following the completion of the relevant experimental trial, samples were centrifuged at 6000rpm/4°C for 10 minutes. The resulting plasma supernatant was drawn off, placed into labelled eppendorfs and stored in duplicate at -20°C until further analysis.

Figure 3.3. Collection of arterialised blood utilizing the heated box method (Clutter et al., 1980).

3.16. Analysis of Lactate and Glucose concentrations

Whole blood lactate and glucose concentrations were determined simultaneously using an YSI 2300 STAT plus analyser (YSI, Yellow Springs Instruments, Yellow Springs, CA, USA). The reaction is generated by an enzyme specific to the relevant substrate, glucose (glucose oxidase), lactate (lactate oxidase), which is immobilised between two membranes. Each membrane contains polycarbonate and cellulose acetate on separate probes. As the substrate is introduced to the membrane layer, hydrogen peroxide is produced. Hydrogen peroxide passes through the cellulose acetate layer and is oxidised, with the generated electron flow proportional to the concentration of substrate (either D-Glucose or L-Lactate).

3.17. Reliability of Lactate and Glucose measurements

The analyser was calibrated automatically each hour against a known standard (1.80g/l and 0.45g/l glucose and lactate respectively). Membranes were routinely updated every 3 months or if the calibration cycle was routinely unable to complete. The coefficient of variation (CV) for lactate and glucose concentrations was determined routinely on a 3 month basis coinciding with membrane alteration. The CV was obtained from the analysis of ten identical samples, both from a resting whole blood sample, and from known standards within a relevant physiological range. For glucose and lactate, the CV remained within a range of 2% and 5%.

3.18. Measurement of pulmonary gas exchange

During each exercise trial, pulmonary gas exchange and minute ventilation values were determined either via breath-by-breath analysis or the Douglas bag technique.

3.19. Breath-by-breath analysis (Fast-response analyser)

Participants wore a nose clip and breathed through a low dead space (35ml), low resistance (< 0.1 $kPa \cdot L^{-1} \cdot s^{-1}$ at 16 $L \cdot s^{-1}$) mouthpiece and volume sensor assembly. Gases were drawn continuously from the mouthpiece through a 2 m capillary line of small bore and analysed for oxygen, carbon dioxide and nitrogen concentrations by a fast-response metabolic analyser (O_2: differential parmagnetic; CO_2: infra-red absorption; Oxycon, Jaeger, The Netherlands). Expiratory volumes were determined using a TripleV turbine volume sensor (Jaeger, The Netherlands).

3.20. Breath-by-breath analysis (Mass Spectrometry)

Participants wore a nose clip and breathed through a low dead space (90ml), low resistance (0.65 cm $H_2O \cdot L^{-1} \cdot s^{-1}$ at 8 $L \cdot s^{-1}$) mouthpiece and turbine assembly (Interface Associates, Laguna Niguel, CA). Gases were drawn continuously from the mouthpiece through a 2 m capillary line of small bore (0.5 mm) at a rate of 600 $ml \cdot min^{-1}$, and analysed for oxygen, carbon dioxide and nitrogen concentrations by a quadrupole mass spectrometer (Airspec QP9000; Clinical and Scientific equipment, Gillingham, Kent, UK).

Each analyser was calibrated before each test using gases of known concentration (British Oxygen Company, UK). The volume sensing device was calibrated using a high-precision 3 L graduated gas syringe (Hans Rudolph Inc, Kansas City, MO, USA) according to the manufacturer's instructions. Multiple syringe strokes were performed at low and high flow rates. Following analog-to-digital conversion, the concentration and volume signals were integrated by personal computer, with account taken of the gas transient delay through the capillary line. Respiratory gas exchange variables ($\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}E$) were calculated and displayed for each breath.

3.21. Average expired air analysis (Douglas bag technique)

Participants wore a nose clip and breathed through a Salford low-resistance respiratory valve/mouthpiece assembly. The mouthpiece was attached to each Douglas bag via a 1m length, 3.75 cm bore Falconia tubing. Expired air was collected into 150 L Douglas bags (Hans Rudolph Inc. Kansas City, Kansas, USA) for a whole number of breaths over a hand timed period. Opening and closing of the Douglas bags was synchronised with inspiration and expiration cycles. Expired air was collected for a minimum of 45 s of each one-minute period.

Expired air was analysed for the percentage of O_2 and CO_2 by sampling through a paramagnetic transducer and an infra-red analyser respectively (Servomex series 1400, Crowborough, UK). Air was sampled for 60 s at a flow rate of 0.9 $L \cdot min^{-1}$. Both gas analysers were calibrated against known gas concentrations prior to each experimental trial (BOC gases, UK). Pure N_2 was

used to calibrate the analysers to 0.00% O_2 and CO_2. Room air was used as the upper range of the calibration cycle. A gas mixture was used to check the linearity of the O_2 meter (14.93% O_2) and to calibrate the range of the CO_2 analyser (5.82% CO_2).

Total expired air volumes were determined by a dry gas volume meter, set at a flow rate of 30 $L \cdot min^{-1}$ (Harvard apparatus ltd, Edenbridge, UK). The gas meter was regularly calibrated using a 7 L graduated gas synringe (Hans Rudolph Inc, Kansas City, Kansas, USA). Each Douglas bag was gently manipulated to expel all collected air. Gas temperature was measured by a thermistor located within the inlet of the volume meter.

3.22. Statistical analysis

All results are reported as Mean ± Standard Error of the Mean (SEM) unless otherwise stated. All statistical analysis was conducted using SPSS version 10. All statistical tests were two tailed with a significance level of 95% confidence. Differences over time were evaluated by two way repeated measures ANOVA. Paired t-tests were employed to highlight differences in mean values within groups between rest and exercise trial conditions. Regression analysis was used to determine correlation between participants and conditions.

CHAPTER IV – THE EFFECT OF INTERDIAN AND DIURNAL VARIATION ON THE MAXIMAL LACTATE STEADY STATE

4.1. Introduction

The maximal lactate steady state (MLSS) has received support as an important measure of endurance capacity, being suggested as the 'gold standard' for assessing individual aerobic capacity (Billat et al., 2003). MLSS has shown sensitivity to training (Billat et al., 2004) leading to its use by researchers and trainers as an athlete assessment and training prescription measure. Whilst the positive outcomes to using MLSS appear apparent, a negative factor is that MLSS classification requires a subject to perform a series of constant workload bouts on successive days making MLSS classification arduous and laboratory intensive (Billat et al., 2003, Billat et al., 2004).

Accordingly, a number of research groups have examined whether it is possible to predict MLSS from single session laboratory assessment (Billat et al., 1994, Billat et al., 1995, Palmer et al., 1999, Harnish et al., 2001, Smith and Jones, 2001, Pringle and Jones, 2002, Dekerle et al., 2003). However it has been demonstrated that many protocols involving single session laboratory tests, suggested to demarcate MLSS, overestimate the work intensity at MLSS (Smith and Jones; 2001; Pringle et al., 2003; Dekerle et al., 2003). It would appear therefore, that the most accurate method to determine MLSS is to perform a series of constant exercise bouts at progressive exercise intensities as originally suggested by Beneke and von Duvillard (1995).

It would seem appropriate that research is conducted to investigate the sensitivity of MLSS to potential laboratory based conditions that might compromise the specific attainment of MLSS. One such condition warranting consideration is the effect that time of day variation may have upon MLSS performance. Circadian rhythms represent alterations in physiological function over a period of ~24 hours (Minors and Waterhouse, 1981). Questioning the relevance of 24-hour observation to physiological performance has led to some authors examining solely diurnal (within day) variation, as this is most applicable to athlete and their coaches (Atkinson and Reilly, 1996b).

The most apparent rhythm documented is that of core body temperature, which displays a peak or acrophase at 18:00h and a nadir at 04:00h (Akerstedt, 1979). It has been suggested that blood lactate responses mirror this fundamental variable of body temperature; however there is limited data investigating this relationship (Atkinson and Reilly, 1996b). The prevalence of a greater exercise capacity occurring in the evening has been well documented for oxygen uptake ($\dot{V}O_2$), ventilation ($\dot{V}E$) and heart rate during sub-maximal (Reilly and Brooks, 1982) and maximal exercise (Davies and Sargeant, 1975, Garrard and Emmons, 1986). More recently, data from our laboratory has shown that time of day variation does not significantly alter the $\dot{V}O_2$ kinetic response during treadmill running (Carter et al., 2002).

With specific regard to diurnal variation and endurance performance markers, Martin and colleagues demonstrated that the lactate and $\dot{V}O_2$ response to an exercise bout, at the running speed corresponding to the lactate threshold (LT), did not alter significantly between morning and evening sessions (Martin et al.,

2001b). Recently Forsyth and Reilly (2004) examined the circadian rhythm of blood lactate concentration during incremental classification of the LT in rowing. Of interest was that these authors used a 4 mM blood lactate value to classify LT. It was observed that the mean power output was significantly lower at 18:00h (220.7 ± 40.7W) than at 02:00h (227.6 ± 24.0W) and that blood lactate was generally elevated at 18:00h (Forsyth and Reilly, 2004). The authors suggested LT detection utilising this method could lead to an inconsistent determination of LT if inter-group values were assessed at different times of day, thereby misrepresenting LT intensity an observation supported by the earlier work of Dalton and co-workers (Dalton et al., 1997).

The use of a 4 $mmol \cdot l^{-1}$ blood lactate reference value (Onset of blood lactate accumulation, OBLA) has previously been suggested as a valid estimate of the work intensity at MLSS by a number of research groups (Stegmann and Kindermann, 1982, Heck et al., 1985, Aunola and Rusko, 1992). This measure derived from the anaerobic threshold (AT) concept, popular with exercise physiologists in the 1980's, was suggested to demarcate the transition where energy provision switched from aerobic to anaerobic energy sources. Subsequently, the terms AT, OBLA and MLSS have been used interchangeably by some research groups to represent the same phenomena. However, the lactate concentration at MLSS can range between 3 and 8 $mmol \cdot l^{-1}$ (Beneke and von Duvillard, 1996) has meant that the reliability of OBLA to estimate MLSS precisely is questionable (Billat et al., 2003),

The classification of MLSS is dependent on an observed change of 1$mmol \cdot l^{-1}$ in lactate concentrations during minutes 10 – 30 of a continuous exercise bout

(Beneke and von Duvillard, 1996). If time of day variation is leading to increased lactate concentrations in the evening when compared to morning sessions, this potentially might compromise the attainment and maintenance of MLSS when performed at varying times of day. Therefore it was the aim of this investigation to assess whether the physiological responses observed at MLSS were sensitive to diurnal (within day) and interdian (day-to-day) variation and to whether any observed differences would have an associated alteration in the classification of MLSS and the physiological responses at this parameter during continuous cycle exercise.

4.2. Methods

4.2.1. Participants

Eight healthy, recreationally active male participants (Mean ± SD; 25 ± 5yr; 178 ± 3.7cm; 74.0 ± 6.9kg) took part in the described study. Participants were recruited if they were currently undertaking endurance activity >3 day week so that any training responses during the present study could be minimised. Participants provided written informed consent after the experimental procedures; associated risks and potential benefits of participation were fully explained. The study was approved by the University of Brighton Ethics Committee.

4.2.2. Laboratory procedures

All participants were familiarised to laboratory procedures prior to testing and were instructed to arrive at the laboratory in a rested and fully hydrated state, having not consumed caffeine or alcohol in the previous 24 h and having

avoided strenuous exercise in the 48 h preceding each test session. After the initial test, diet was recorded for the following three days and then replicated before subsequent test sessions. Participants reported to the laboratory on a total of eight occasions over a period of four weeks. During this period they completed an incremental test to exhaustion to determine Lactate threshold (LT) and $\dot{V}O_2$max, a series of 30 min constant load trials to determine the power and physiological responses at MLSS; and four further repetitions of the power corresponding to MLSS, twice more at 18:00h and once at 12:00h and 06:00h.

4.2.3. Interdian and Diurnal interventions

Following classification of MLSS, each subject returned to the laboratory on four more occasions, each separated by at least 24 h rest, to determine interdian and diurnal variation. Following a randomised design, the intensity at MLSS was replicated on two more occasions at 18:00 to examine interdian variation and once at 06:00h and 12:00h to determine diurnal variation. During these trials, core body temperature was continuously recorded. To allow for continuous measurement of rectal temperature, a thermistor probe was inserted approximately 10 cm beyond the anal sphincter. Readings of rectal temperature were taken from a single channel data logger (ET401, Libra Medical, Reading, UK).

Two hours before each test session participants consumed a light snack (~160 kcal: 30g CHO, 2.5g Protein, 3.5g FAT) to standardise diet (Carter et al., 2002). Prior to the 06:00h trial participants were instructed to wake at 04:00h and follow the same protocol. The participants were instructed to maintain their

normal habitual physical activity during the three week experiment, whilst the diurnal trials were randomised in an attempt to minimise individual sleep-waking cycle.

4.2.4. Statistical analysis

Carbohydrate (CHO) and lipid oxidation rates were calculated from gas exchange measurements according to the non-protein respiratory quotient technique as used by MacRae et al., (1995). CHO oxidation rate was calculated by the following equation.

$$\text{CHO oxidation rate} = 4.585 - 3.22 \cdot \dot{V}CO_2 - 3.22 \cdot \dot{V}O_2$$

A two-way ANOVA was used to assess differences between mean group responses, either across the three time domains or replicate trials at the same time of day. Regression analysis was used to examine correlation between changes in body temperature and exercise duration. The calculated rate constant for temperature increase (k) was determined from a plot of the log rectal temperatures over time. Results are presented as the mean ± SEM.

4.3. Results

4.3.1. Interdian variation

There were no differences found in physiological responses measured at MLSS across the three test sessions (Table 4.1). Mean lactate taken between minutes 10-30 during MLSS ranged from 5.7 ± 0.1 $mmol \cdot l^{-1}$ (TRIAL 1), 5.9 ± 0.1 $mmol \cdot l^{-1}$ (TRIAL 2) and 5.5 ± 0.1 $mmol \cdot l^{-1}$ (TRIAL 3). Heart rate across the three test sessions ranged from 167 ± 1 $beats \cdot min^{-1}$ (TRIAL 1), 167 ± 2 $beats \cdot min^{-1}$ (TRIAL 2) to 167 ± 1 $beats \cdot min^{-1}$ (TRIAL 3).

Table 4.1. Interdian variation of physiological responses at the MLSS between minutes 10 and 30 (mean ± SEM)

Variable	Trial 1	Trial 2	Trial 3
Blood Lactate ($mmol \cdot l^{-1}$)	5.7 ± 0.1	5.9 ± 0.1	5.5 ± 0.1
% VO_2max	78 ± 0.3	79 ± 0.3	78 ± 0.3
RER	0.93 ± 0.01	0.94 ± 0.01	0.93 ± 0.01
Heart rate ($beats \cdot min^{-1}$)	167 ± 2	167 ± 3	167 ± 2
VO_2 ($l \cdot min^{-1}$)	3.3 ± 0.1	3.4 ± 0.1	3.3 ± 0.1
VE ($l \cdot min^{-1}$)	100 ± 2.2	1.01 ± 2.6	100 ± 2.2

4.3.2. Diurnal variation

All eight participants completed a trial at MLSS at three time points, referred to as AM (06:00h), MID (12:00h) and PM (18:00h). There were no differences found between the AM and MID trials, or the MID and PM trials so for clarity of reading the MID trial data will be omitted for subsequent interpretation and discussion.

Clear diurnal variation was observed in core body temperature values between AM and PM conditions both at rest (P<0.01) and throughout each MLSS session (P<0.01, figure 4.1). Resting mean core temperature was lower in AM (36.83°C) compared to PM (37.48°C) (P<0.01). Core temperature was consistently higher during the PM session, increasing by 3% during 30 min at MLSS to 38.63°C. This was compared to the 3.4% increase during the AM trial with end temperature recorded as 38.11°C. There were no significant changes in the rate constant for temperature increase across time of day conditions. The calculated rate constants for temperature increase were k=4.0 ± 0.43 x 10^{-3} °C min^{-1} during PM and k=5.0 ± 0.53 x 10^{-3} °C min^{-1} during the AM trials (figure 4.2).

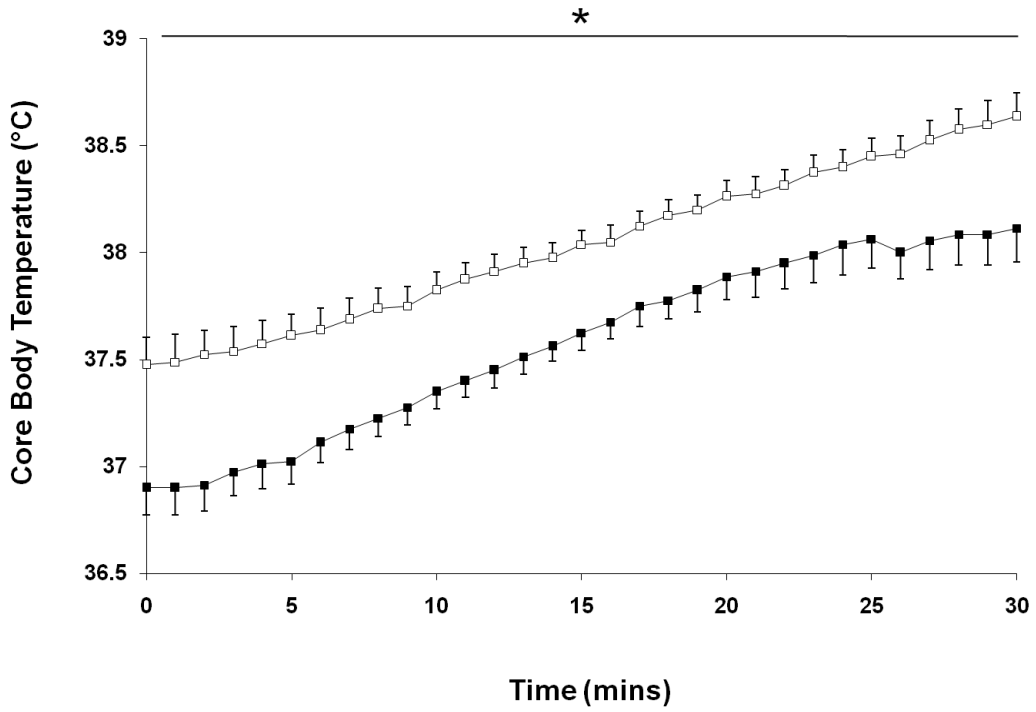

Figure 4.1. Mean core body temperature during 30min cycling at MLSS. Dark squares represent PM trial, open squares represent AM trial (* *P*<0.05)

Mean lactate concentrations at MLSS were lower at each time point during the AM trial compared to the PM trial (P<0.05) (Figure 4.3). There were clear differences observed across the time conditions with mean lactate concentrations (10-30min) at AM 4.88 ± 0.02, at MID 5.69 ± 0.07 and at PM 6.14 ± 0.07 $mmol \cdot l^{-1}$ (P<0.05) (figure 4.4).

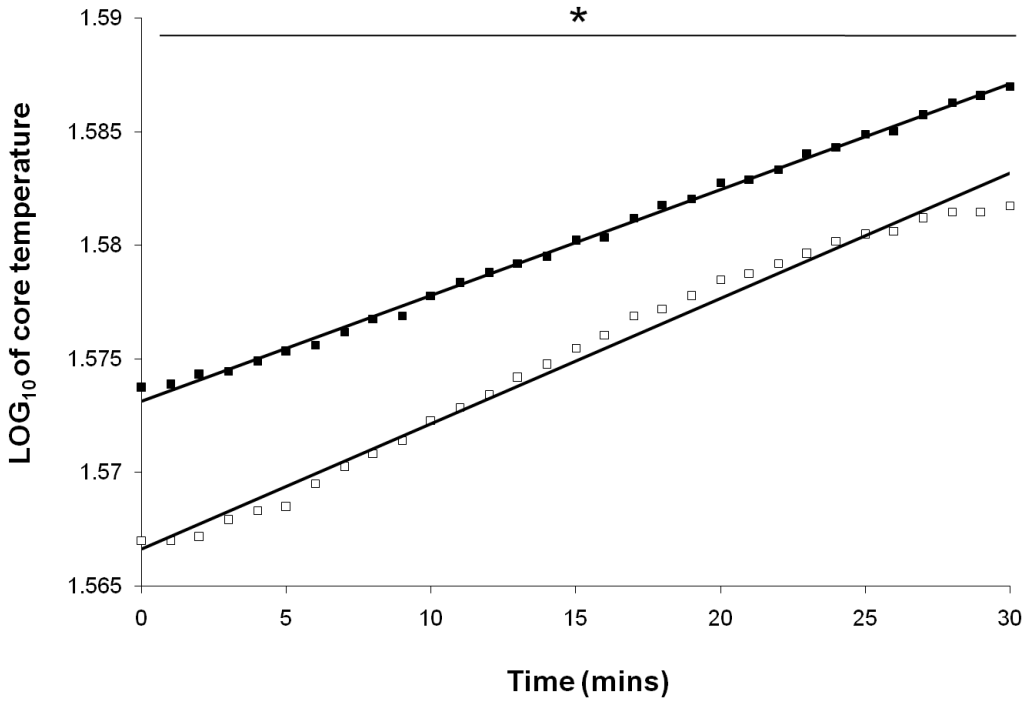

Figure 4.2. Calculated rate constant for core temperature increase during 30 min cycling at MLSS. Dark squares represent PM trial, open squares represent AM trial (* P<0.05)

There were no differences found in RER data across the time domains. Mean RER values taken between 10 and 30 min at MLSS were 0.93 ± 0.004 at AM, 0.93 ± 0.02 during MID and 0.93 ± 0.002 at PM. CHO utilisation showed a trend to be higher during the PM trial accounting for 79.64 ± 0.94% total energy provision when compared to 76.25 ± 1.26% during the AM trial, however this trend was not statistically significant (figure 4.5).

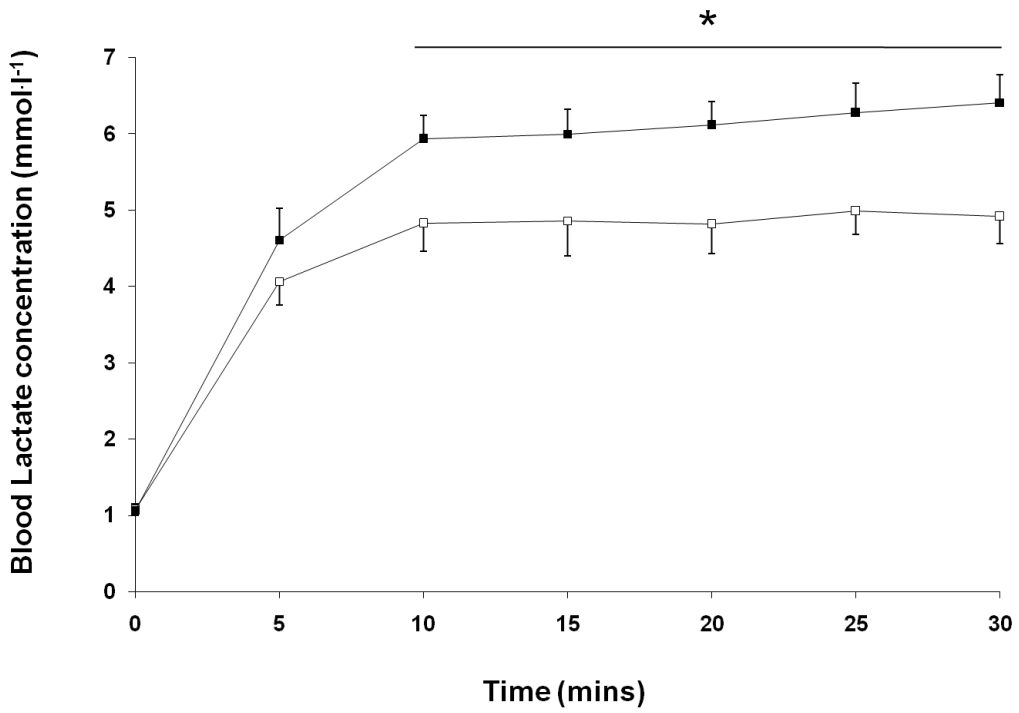

Figure 4.3. Mean blood lactate response during 30 minutes cycling at MLSS. Dark squares represent PM trial, open squares represent AM trial (* *P*<0.05)

There were no differences found in absolute $\dot{V}O_2$ values across time of day. Mean $\dot{V}O_2$ values taken between minutes 10 and 30 at MLSS were 3.34 ± 0.01 $l \cdot min^{-1}$ at AM, 3.20 ± 0.01 $l \cdot min^{-1}$ during MID and 3.33 ± 0.02 at PM. There was no change in responses when $\dot{V}O_2$ was expressed relative to power output with mean values between 10 – 30 mins at MLSS 15.2 ± 0.1 $ml \cdot W^{-1}$ AM and 15.1 ± 0.1 $ml \cdot W^{-1}$ PM respectively (Table 4.2).

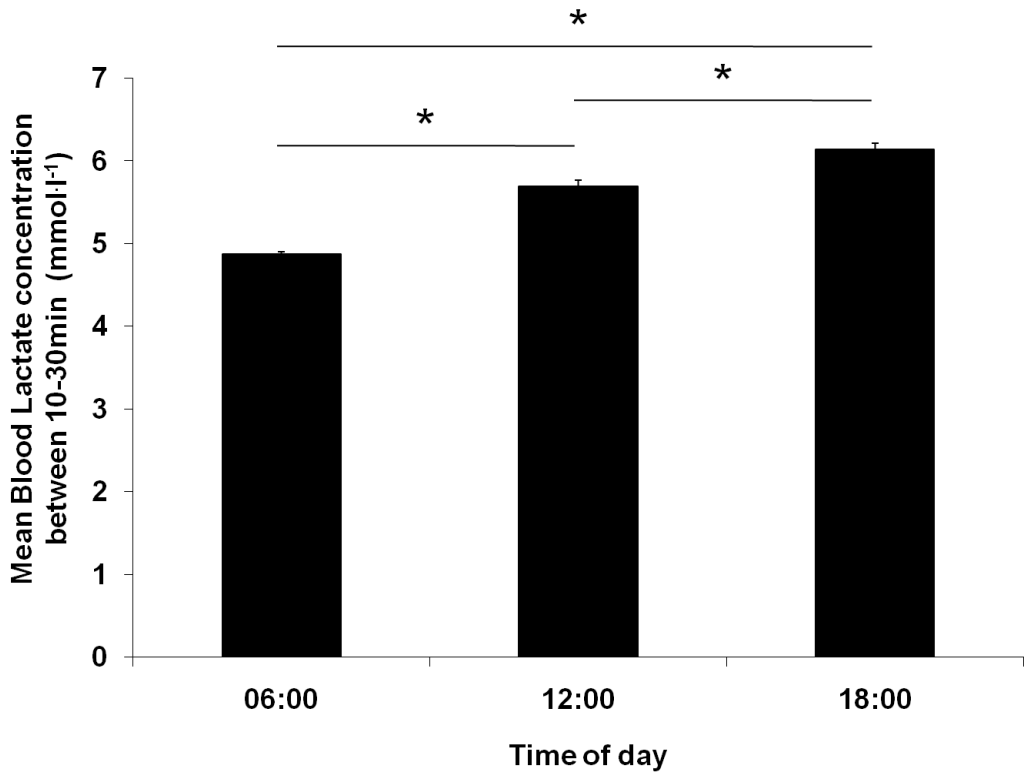

Figure 4.4. Mean blood lactate concentrations across time domains between 10 and 30 minutes at MLSS (*P<0.05)

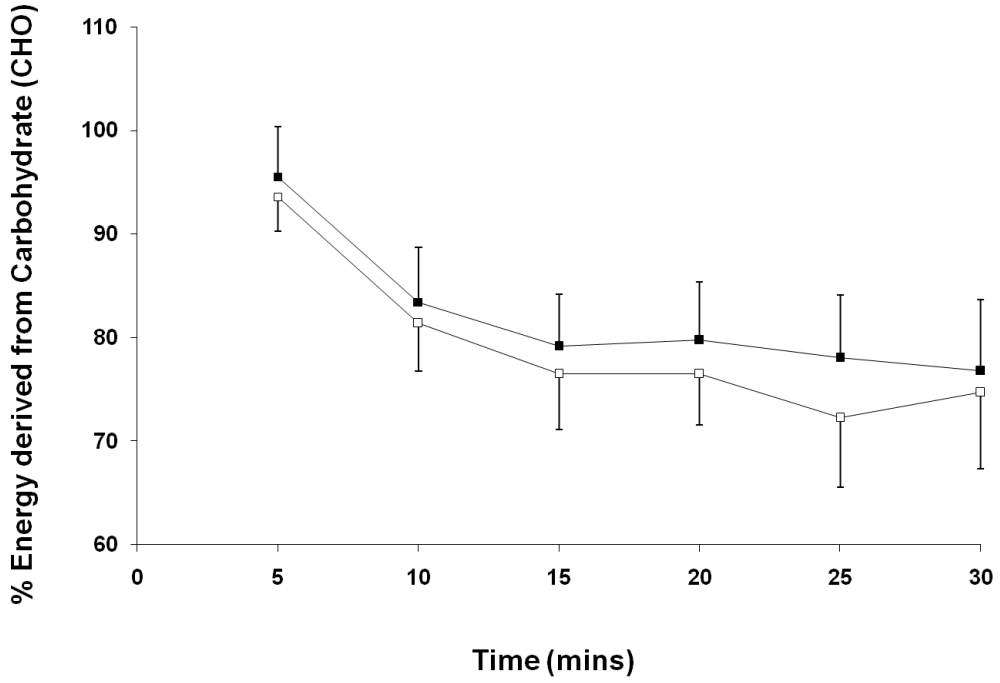

Figure 4.5. Percentage of energy derived from carbohydrate (CHO) during 30 minutes cycling exercise at MLSS. Dark squares represent PM, open squares represent AM (*P<0.05)

4.4. Discussion

Diurnal rhythms have been observed to influence many aspects of human metabolism, which are thought to primarily be governed by the fundamental circadian rhythm of body temperature (Atkinson and Reilly, 1996). The results presented here showed a clear diurnal variation in blood lactate concentrations at MLSS (Figure 4.3 and 4.4). The blood lactate response was shown to parallel that of core body temperature which was consistently higher during the PM trials compared to AM. This observation is in agreement with other studies (Reilly and Brooks, 1982; Martin et al., 2001); however is the first to display such a response at MLSS. There were no other notable physiological responses altered by diurnal effects suggesting that the variation in body temperature may have had a direct effect on lactate production and clearance mechanisms.

Interdian examination showed that the physiological responses at MLSS were repeatable without alteration in the classification of this marker (participants remained within the 1 $mmol \cdot l^{-1}$ alteration criteria used to establish MLSS) (Table 4.2). This data adds confidence to the prescription of MLSS for training and laboratory based assessment. If a subject is required to perform a series of exercise bouts at MLSS across a series of days, our data would suggest that the individual would maintain a steady state response in each of the training sessions provided they were conducted at the same time of day. Further, demonstration of a consistent heart rate response at MLSS also suggests that this method of monitoring performance could be of potential benefit during non-laboratory based sessions (e.g. field type).

Whilst the MLSS has received support as an important marker of endurance performance, there still remain unanswered questions as to the repeatability of this measure. It has previously been reported that inter-individual differences in lactate concentrations can range from 3 to 8 $mmol \cdot l^{-1}$ at MLSS (Beneke and von Duvillard, 1996), whilst varying with age (Beneke et al., 1996; Mattern et al., 2003) and training status (Carter et al., 2000; Billat et al., 2004). The present study was designed therefore, to examine whether variability exists when the intensity at MLSS is repeated across days and across time of day, thereby assessing the effect of interdian and diurnal variability respectively.

It is important to reiterate, that although clear differences were observed in mean lactate concentrations, the steady state at MLSS was not affected by diurnal variation, so therefore we can assume that balance between lactate production and removal existed. There are a number of possible explanations as to why a reduction in steady state lactate was observed during the AM exercise bouts. Firstly, alterations in body temperature may have increased glycolytic rate so that an increased rate of lactate appearance occurred in the evening trials. Or secondly, that temperature had an effect on oxidation of lactate so that clearance processes were affected. Future kinetic examination of lactate flux during AM and PM exercise sessions could address this question. A further possible explanation of the altered lactate response with time of day could be due to an altered catecholamine response. Unfortunately we do not have data regarding circulatory catecholamine concentrations during each trial as this was beyond the scope of this investigation, however research has suggested involvement in catecholamine release in the maintenance of a MLSS and so this issue would clearly warrant further investigation (Baron et al., 2003).

Martin and colleagues (Martin et al., 2001a) have demonstrated that RER during running at LT is reduced in the morning, suggesting a preference to fat oxidation during this time period. Whilst the present study found no significant alteration in RER across time domains, there was a trend towards increased fat oxidation during the AM trials when calculating the relative percentage of energy derived from carbohydrate and lipid stores. This observation could potentially explain the lower lactate levels at MLSS during the AM exercise bouts, whilst demonstrating that fuel use can be adapted for ATP provision at MLSS intensity. Average RER values at MLSS have been reported as ~1.00 throughout 60 min exercise (Billat et al., 2004) however there is no kinetic data to quantify fuel utilisation at MLSS. Alternatively the elevated body temperature during PM exercise bouts could have led to increased glucose turnover due to up-regulation of glycolytic enzyme activity; however we have insufficient data to comment on this issue.

With regard to lactate transport temperature sensitivity, Roth and Brooks (1990) demonstrated that initial rates of L(+)-Lactate uptake were threefold higher at 37°C compared to 25°C in rat sarcolemmal membrane vesicles. Further, Watt et al., (1994) confirmed that contraction *per se* had no effect on lactate transport in perfused rat muscle; therefore, the observed differences could not be attributed to the exercise undertaken. The elevated lactate concentrations observed during the PM trials in the present study occurred without increase in the cost of exercise as there was no change observed in $\dot{V}O_2$ values. Therefore, lactate transport may have been upregulated by increased temperature to maintain a steady state, whilst the fact that steady state balance was not compromised suggests that both production and removal systems were

equally affected. The higher lactate concentrations during the PM trial could potentially be due to a change in the michaelis constant (Km) for one of the processes in removal or appearance or a Vmax effect for lactate transport proteins (Coles et al., 2005).

Our data suggests a number of considerations should be taken when MLSS laboratory based testing takes place. Firstly the use of the 4 mmol·l^{-1} reference value for estimating MLSS should be reconsidered due to the clear inter-individual variation amongst MLSS concentrations, and the observed diurnal variation in lactate concentrations at MLSS displayed within this study. Further, care should be given when incremental sessions and constant load exercise test sessions are performed at differing times of day to minimise the risk of diurnal effects. The present study has demonstrated MLSS to be robust to multiple test sessions across days, allowing confidence of replication during a training design, or repeated lab testing. The variation we showed in MLSS across time domains does lead to the question as to whether these alterations could have a subsequent effect to training adaptation if the exercise bout is performed at different time points in a day.

There is limited data assessing the optimum time of day for sub-maximal training bouts. Torii et al. (1992) suggested that four weeks of cycle ergometer training (4 x 30 min session at 60% $\dot{V}O_2$max) was more beneficial when exercise was performed in the afternoon (15:00) compared to morning (09:00) or evening (20:00) sessions (Torii et al., 1992). Unfortunately, this data was compromised due to the afternoon data set not being fully complete pre and post lab testing, therefore allowing little support for time of day differences.

Dalton et al., (1997) have provided contrasting data suggesting that circadian rhythms have no effect on team trial cycling performance of 15 min duration. The authors suggested that although athletic performance may be enhanced by training programs that are compatible with an individual's body clock, the ability to perform and train at various times has an adaptive response which appears to over-ride these natural inherent rhythms (Dalton et al., 1997). It has however been demonstrated that a circadian specificity to exercise training does exist, with Hill and colleagues (1989) showing that participants who trained in the morning had relatively higher post-training thresholds in the morning, whilst participants who trained in the afternoon displayed a similar trend (Hill et al., 1989). A recent study by Edwards et al. (2005) suggested that a moderate exercise session at the same time of day, on the day prior to competition benefited performance (Edwards et al., 2005). The authors concluded that a phase-shifting effect of exercise or habit might occur allowing the athlete to synchronize to the time of competition. There is a dearth of research investigating the effect of training on MLSS. Billat et al. (2004) have demonstrated that specific training at MLSS improves running performance and time to exhaustion at this marker. What needs to be ascertained is whether the diurnal variation of MLSS could affect the training induced adaptations of this marker if exercise bouts within a training program are performed across time domains.

In summary, the results of the present study demonstrate that MLSS is replicable across test sessions at the same time of day making it a valid laboratory based measure, however there was an observed alteration in the lactate concentration at MLSS following diurnal variation. The variation in lactate concentration paralleled the fundamental circadian rhythm of core body temperature, and was attributed primarily to an increase in

fat oxidation during the AM trials. Further research is needed to examine the exact mechanisms responsible for this observation, and whether this alteration affects the training induced response at MLSS when training bouts are performed at differing times of day. Finally, our data supports the synchronisation of lab testing and training sessions to the same time domain. Further, given the fluctuation in the blood lactate response at MLSS, the data presented would question the efficacy of a blood lactate reference value to estimate the MLSS.

CHAPTER V – EXAMINING THE EFFICACY OF THE RUNNING SPEED AT THE MAXIMAL LACTATE STEADY STATE AS A TRAINING STIMULUS

5.1. Introduction

The blood lactate concentration sustainable at the MLSS has been shown to vary considerably (3-8 $mmol \cdot l^{-1}$) across participants (Beneke, 1995, Pringle, 2002), whilst being sensitive to training (Billat, 2004) and influenced by age (Mattern et al., 2003). The exercise intensity at MLSS has been shown to be sustainable for ~60 min (Lajoie et al., 2000, Billat, 2004) making it an attractive exercise intensity for training prescription or for events over this time period. The potency of using the running speed at MLSS, itself, as a training stimulus has been demonstrated recently by Billat and colleagues (Billat, 2004) who measured an increase in both time (from 44 to 63 min) and distance (from 10 to 16 km) sustained at the MLSS following six weeks of training (2 sessions per week) at a velocity corresponding to an individual's MLSS. This training stimulus also increased maximal and sub-maximal markers of aerobic capacity of these individuals by ~4%. The fact that two sessions per week could produce such adaptation is interesting as it would allow the inclusion of such training sessions into an active training program, without causing excessive disruption to the whole training design.

Traditionally there are methodological approaches with regard to endurance training. Firstly, an increase in the duration of exercise leads to a greater adaptive response, and secondly that an increase in intensity of exercise also elicits adaptation (Dudley et al., 1982). To examine the relationship between

increased training intensity and performance gains, Weltman et al., (Weltman et al., 1992) prescribed training above and below the lactate threshold (LT) in 24 previously untrained women. Whilst training above and below this marker showed an increase in the LT, it was suggested that for continued improvement, some aspect of the training must be conducted above the LT. The data provided by Weltman et al., (Weltman et al., 1992) added additional support to the previous research of Acevado and Goldfarb (Acevedo and Goldfarb, 1989) that demonstrated a beneficial effect of intermittent training above and below 10km race pace in a group of well trained runners. The performance gains observed in this study were attributed to the repetitions performed above average race pace creating a larger training stimulus than continuous training at 10km exercise intensity.

Whilst the performance benefits of training at the MLSS running speed have been clearly shown by Billat et al., (Billat, 2004), a number of questions still remain regarding the use of the MLSS as a training stimulus. First, would it be more beneficial to train at the MLSS or at exercise intensities surrounding this marker to improve aerobic performance? Secondly, Billat et al., (Billat, 2004) demonstrate the use of the MLSS as a training stimulus in master endurance athletes. Therefore, establishing whether training at the MLSS produces a similar effect in participants with different fitness profiles could be useful for future use of the MLSS as a training stimulus.

Therefore the intention of this study was to examine the use of the MLSS as a training stimulus during an eight week period. Two groups, completing either continuous (CON) running, at an exercise intensity corresponding to the MLSS

or intermittent (INT) training of the same average workrate, but 0.5km·h^{-1} above and below the MLSS completed the eight week training period. Based on previous research (Acevedo and Goldfarb, 1989, Weltman et al., 1992) it was hypothesised that compared to continuous training, an intermittent training approach of the same average intensity would elicit a larger training response due to the added stimulus provided by the periods of time spent above MLSS.

5.2. Methods

5.2.1. Participants

Fourteen participants volunteered to take part in the study (12 males, 2 females; age 25 ± 6 years, height 176 ± 5 cm, body mass 76 ± 8 kg mean ± SD). Participants were familiar with treadmill running, running to volitional exhaustion and laboratory exercise testing procedures. Prior to any testing, participants gave written, informed consent after the experimental procedures; training protocol, associated risks and benefits of participation were fully explained. The procedures were approved by the University of Brighton Ethics Committee.

5.2.2. Experimental Procedures

Participants were instructed to arrive at the laboratory in a rested and fully hydrated state, three hours post-prandial, having avoided strenuous exercise in the 48 h preceding the test session. The exercise tests were completed over two, ten day periods at the start and finish of the training period for both training groups. For each subject, tests took place at the same time of day (±2 h) to minimize the effect of diurnal biological variation (Atkinson and Reilly, 1996a).

Participants were asked to complete food diaries on the two days before attending the laboratories, and to replicate this diet before each session. Prior to a trial commencing, participants completed a 5 minute self-paced warm-up. Each subject then completed: an incremental treadmill test to determine individual lactate threshold and $\dot{V}O_2$max values and a series of constant-speed treadmill sessions to determine the running speed at the MLSS.

5.2.3. Control Period

Following completion of the initial testing period, all participants underwent a 4 week control period where they were asked to continue their habitual activity levels. During this time, participants were provided with training diaries to record all physical activity undertaken, and instructed to continue habitual activity levels. At the completion of the 4 week period participants returned to the laboratories and MLSS was re-classified (see following text) to determine if regular activity had any influence on the previously characterized markers of endurance capacity.

5.2.4. Training design

After the 4 week control period, participants started 8 weeks of endurance exercise training. Participants were pair matched for $\dot{V}O_2$max and randomly assigned into either a continuous (CON) or intermittent (INT) training group. Participants were required to perform 2 exercise sessions per week, increasing from 21 min to 33 min over the eight-week period. Table 5.1 describes the training undertaken and shows the increases in training duration and the difference in training design between the two groups. Each training session took place in the laboratories at the University of Brighton under supervision by an experimenter. Heart rate was recorded throughout (Polar Electro, Kempele, Finland).

Table 5.1. Training program design for continuous (CON) and intermittent (INT) training groups

Week	Frequency and Duration	Training Design
1 and 2	2 x 21 min	(CON) – 21 min (INT) – 7 x 3 min stages.
3 and 4	2 x 27 min	(CON) – 27 min (INT) – 9 x 3 min stages.
5, 6, 7 and 8	2 x 33 min	(CON) – 33 min (INT) – 11 x 3 min stages.

For the continuous training sessions, the participants exercised at the MLSS. The intermittent training regime comprised of a 5 minute warm-up, followed by 3 minute stages at 0.5 $km \cdot h^{-1}$ above and below the MLSS. This design of training meant that the mean distance covered during training was equal for the two training groups. All participants completed training diaries during the 8 week training period, and were asked to replicate the exercise pattern they followed during the control period, including the prescribed training, so that the type of training altered as opposed to training frequency. Heart rate training data from individual participants was monitored on a weekly basis to ensure that all participants conformed to the training protocol.

5.2.5. Statistical analysis

Results are reported as mean ± SEM. Repeated measures ANOVA (SPSS version 12) were used to determine individual differences between pre-training and post training values and the mean group responses. Statistical significance was accepted at $P<0.05$ for all of the comparisons between measures.

Regression analysis was conducted to examine the correlation between measures of endurance capacity pre and post training.

5.3. Results

5.3.1. Training compliance

Training adherence was 100%, all participants completed the 16 prescribed training sessions. Training diaries showed participants maintained physical activity levels as observed during the control period, with the only alteration in training during the 8 week training period being the prescribed training sessions.

5.3.2. Effect of training on the maximal lactate steady state

There were no significant differences in the physiological responses measured between the training groups. Body mass remained constant throughout the training period and was not significantly altered by the training.

Running speed at the MLSS increased in CON by 8% from 12.3 ± 0.6 to 13.4 ± 0.6 $km \cdot h^{-1}$ (P<0.05) and in INT by 5% from 12.2 ± 0.7 $km \cdot h^{-1}$ to 12.9 ± 0.7 $km \cdot h^{-1}$ (Figure 5.1; P<0.05). The blood lactate concentrations at the MLSS (Pre training: 3.9 ± 0.1 $mmol \cdot l^{-1}$ and 3.3 ± 0.4 $mmol \cdot l^{-1}$ in the CON and INT groups respectively) were significantly lower (P<0.05) at the same relative speed post training in both groups. At the new MLSS, lactate was significantly higher than before training (P<0.05) at 4.6 ± 0.5 $mmol \cdot l^{-1}$ and 3.7 ± 0.6 $mmol \cdot l^{-1}$ in CON and INT respectively (Figure 5.2).

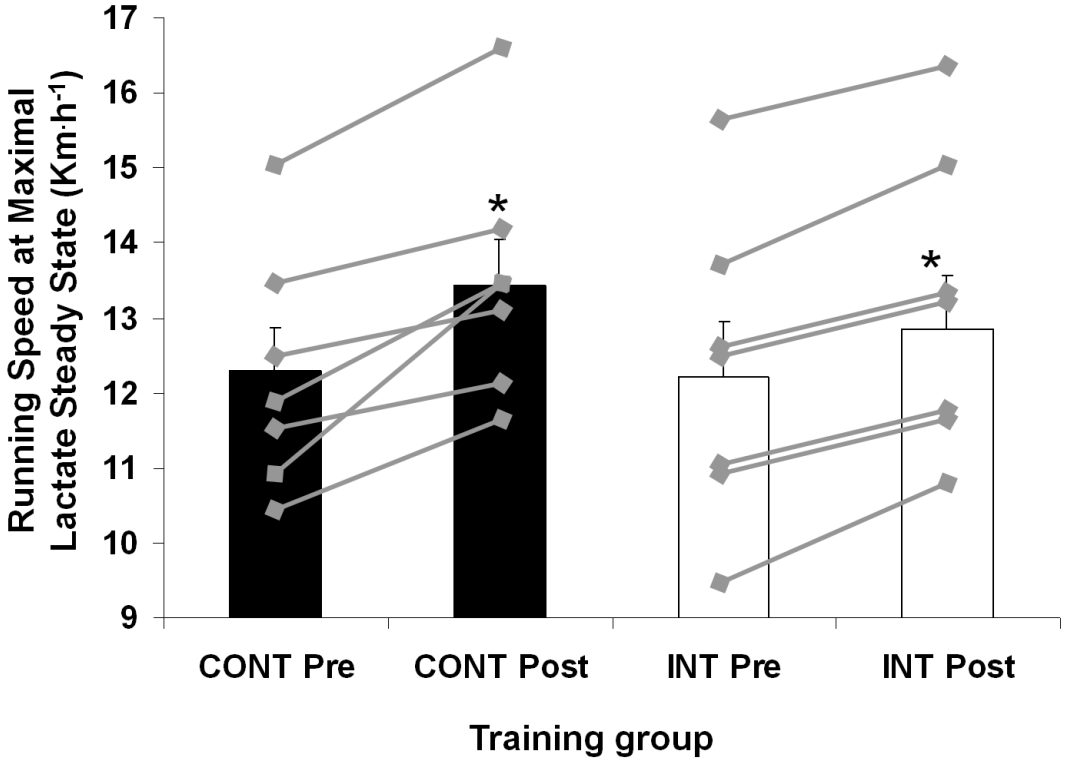

Figure 5.1. Mean and individual improvements in running speed at MLSS pre and post training. Lines represent individual changes pre and post training. ■ Continuous training group, □ Intermittent training group. *difference between pre and post (*P*<0.05)

The $\dot{V}O_2$ and $\%\dot{V}O_2$max at MLSS were reduced at the same relative speed post- training in both CON and INT groups (*P*<0.05). When expressed relative to body mass and running velocity the $\dot{V}O_2$ response showed a similar trend (Table 5.2). Following training, the RER at the pre-training MLSS (10-30 min) was reduced from 0.91 ± 0.1 to 0.86 ± 0.01 in CON and 0.91 ± 0.01 to 0.88 ± 0.01 in INT. At the new MLSS RER had returned to pre training values in both groups. The percentage of energy derived from CHO during exercise at MLSS was 68 and 71% in CON and INT pre training, reduced to 55 and 60% post training and increased to 92 and 89% at the new MLSS in CON and INT respectively (Table 5.2).

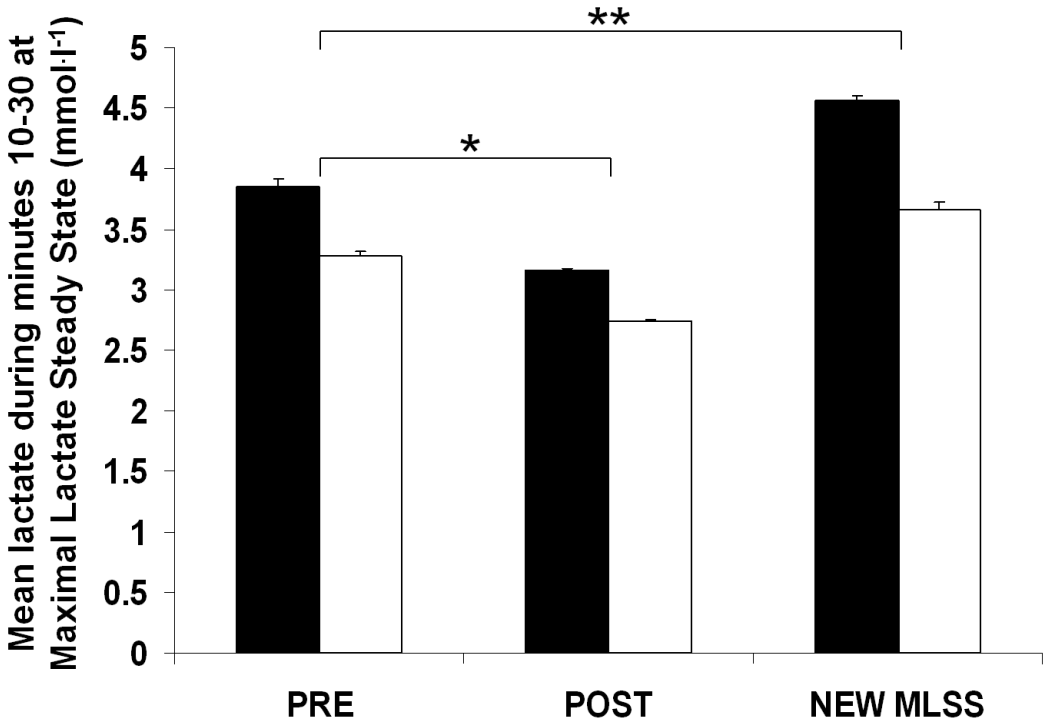

Figure 5.2. Mean lactate response between 10 – 30 minutes at the MLSS. ■ Continuous training group, □ Intermittent training group. *difference between pre and post ** difference between pre and new MLSS (*P*<0.05)

HR at the MLSS was reduced at the same relative speed post training in both CON and INT groups (*P*<0.05) and returned to pre training levels at MLSS post training. The average HR (10-30min) was 178 ± 1 beats·min^{-1} before training 166 ± 1 beats·min^{-1} post training and 180 ± 1 beats·min^{-1} at the new MLSS in CON, whilst 170 ± 2 beats·min^{-1} pre training 159 ± 1 beats·min^{-1} post training and 173 ± 2 beats·min^{-1} at the new MLSS in INT.

Table 5.2. Changes in physiological variables at the MLSS following eight weeks continuous (CONT) and intermittent (INT) training

Variable		Pre-training	Post training	New MLSS
vMLSS ($km \cdot h^{-1}$)	CON	12.3 ± 0.6	-	13.4 ± 0.6**
	INT	12.2 ± 0.7	-	12.9 ± 0.7**
% $\dot{V}O_{2max}$ at MLSS	CON	86.2 ± 0.3	78.7 ± 0.5*	86.7 ± 0.6
	INT	83.9 ± 0.4	76 ± 0.4*	82.8 ± 0.1
$\dot{V}O_2$ ($L \cdot min^{-1}$) MLSS	CON	3.45 ± 0.12	3.05 ± 0.16*	3.56 ± 0.19
	INT	3.32 ± 0.20	3.14 ± 0.77*	3.47 ± 0.34
RER MLSS	CON	0.90 ± 0.01	0.86 ± 0.01*	1.00 ± 0.01
	INT	0.91 ± 0.01	0.88 ± 0.01*	0.97 ± 0.01
CHO Utilization (%)	CON	68.2 ± 1.18	55.5 ± 1.9*	91.8 ± 0.5**
	INT	71.8 ± 0.5	60.6 ± 1.2*	88.8 ± 0.9**
HR MLSS ($Beats \cdot min^{-1}$)	CON	178 ± 1.6	166 ± 1.1*	180 ± 1.5
	INT	170 ± 1.6	159 ± 0.7*	173 ± 1.5

5.3.4. Effect of training on parameters of endurance performance

$\dot{V}O_2$max increased in CON by 10% and in INT by 6% (P<0.05). Running speed at $\dot{V}O_2$max increased in CON by 5% and in INT by 7% (P<0.05) (Table 5.3). Running speed at the LT increased in CON by 7% and in INT by 9% (Figure 5.3; P<0.05). $\dot{V}O_2$ at LT was reduced in CON by 2% from 2.5 ± 0.2 to 2.3 ± 0.2 $L \cdot min^{-1}$ (P<0.05) and reduced in INT by 11% from 2.7 ± 0.2 to 2.4 ± 0.2 $L \cdot min^{-1}$ (P<0.05). The % $\dot{V}O_2$max at LT was reduced in CON by 10% and in INT by 16% (Table 5.3).

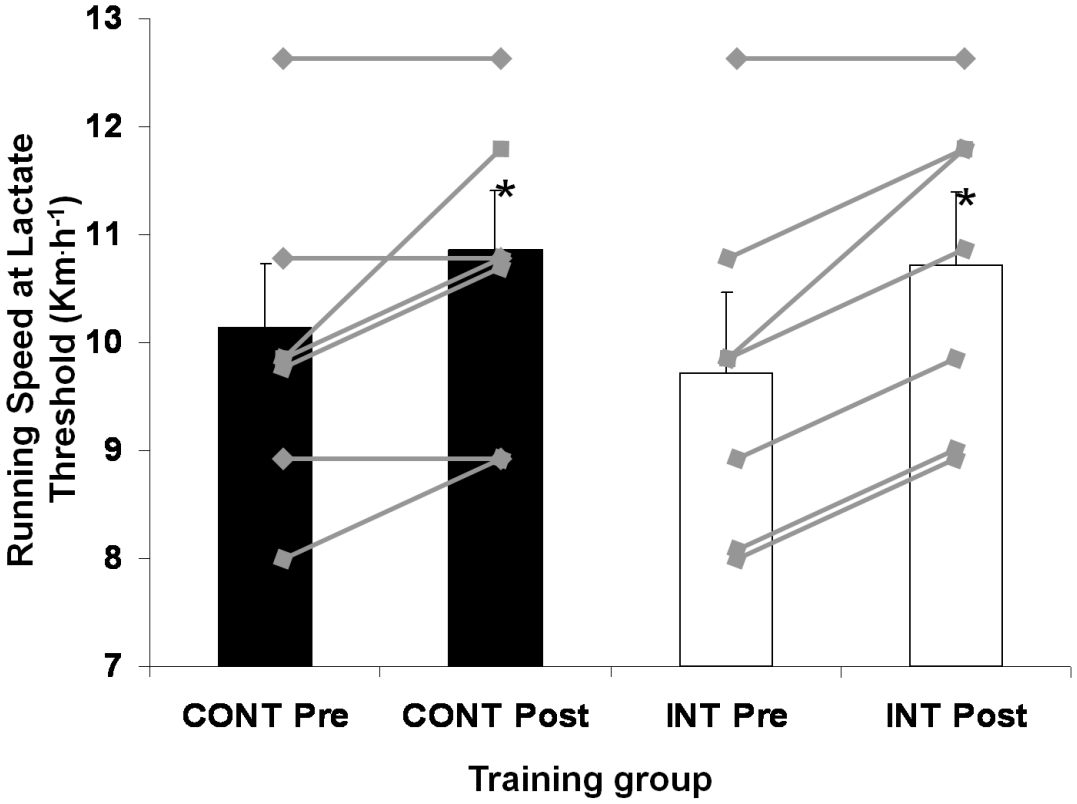

Figure 5.3. Mean and individual improvements in running speed at LT pre and post training. Lines represent individual changes pre and post training. ■ Continuous training group, □ Intermittent training group. *difference between pre and post (*P*<0.05)

Running speed at MLSS was shown to correlate significantly with running speed at LT (R^2 = 0.73) and $\dot{V}O_2$max (R^2 = 0.76) pre training indicating association between these three endurance markers (*P*<0.05). This correlation was maintained following training with MLSS correlating with LT (R^2 = 0.65) and $\dot{V}O_2$max (R^2 = 0.71; *P*<0.05).

Table 5.3. Changes in parameters of aerobic capacity following eight weeks continuous (CONT) and intermittent (INT) training

Variable		Pre-training	Post training	% Change
vLT(km·h^{-1})	CON	10.1 ± 0.6	10.8 ± 0.6 *	7
	INT	9.7 ± 0.7	10.7 ± 0.7 **	9
% $\dot{V}O_{2max}$ at LT	CON	68.0 ± 4.3	65.4 ± 4.2 *	-3
	INT	70.7 ± 4.3	65.1 ± 6.3 *	-7
$\dot{V}O_{2max}$ (L·min^{-1})	CON	3.8 ± 0.2	4.2 ± 0.3 **	10
	INT	3.9 ± 0.3	4.2 ± 0.2 *	7
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	CON	49.6 ± 1.5	54.9 ± 3.7 *	10
	INT	52.5 ± 3.6	56.1 ± 2.8 *	7
v$\dot{V}O_{2max}$ (km·h^{-1})	CON	17.0 ± 0.7	17.9 ± 0.6 *	5
	INT	16.7 ± 0.9	18.0 ± 0.7 *	7
Body Mass (Kg)	GROUP	76.1 ± 9.8	76.1 ± 9.8	-

5.4. Discussion

The principal finding of this study was the identification that training with continuous or intermittent regimes at an intensity, based on MLSS, can improve running speed at this marker, as well as providing adaptation for a host of other endurance variables (i.e. LT, $\dot{V}O_2$max and running economy). Running speed at MLSS increased by 8% in the continuous training group and by 5% in the intermittent training group, however there were no significant differences between groups. A secondary finding of the present study was the observation that improvements in MLSS following training are independent of fitness as a similar increase in running speed at MLSS was observed in participants of

varying initial $\dot{V}O_2$max levels. Further the improvement in MLSS running speed was complemented with improvements in running speed at LT and $\dot{V}O_2$max, indicating the positive effect of this training mode on maximal and sub-maximal aspects of endurance capacity (Table 5.2).

Our original research hypothesis was that an intermittent style of training 0.5 $km \cdot h^{-1}$ above and below the MLSS would elicit greater adaptation than continuous training at the MLSS, in a similar manner to that previously described by Acevado and Goldfarb (Acevedo and Goldfarb, 1989) for 10 km running performances in elite male runners. The design of the INT training group was chosen for a number of reasons. Firstly, it was our aim to modify the training bout intensity without dramatically altering the type of training (i.e. high intensity intervals). Secondly, we wanted to keep the duration of each training bout the same for each group, so interval repetitions exceeding 0.5$km \cdot h^{-1}$ would have made this difficult. And thirdly, running speed at the MLSS was the basis for our training stimulus so the INT design allowed us to examine the use of MLSS in a different manner to previous research (Billat, 2004). Our results show that this form of training is beneficial in mediating improved performance (5% improvement in the MLSS), but contrary to our hypothesis was not as beneficial as continuous training at the MLSS (9% improvement in MLSS). Our data adds support to the suggestion that the best way of increasing running velocity at MLSS may be to specifically train at this intensity, however comparison of our results to other forms of training (i.e. high intensity interval or ramp training) is required before this statement can be categorically accepted.

The reduced lactate concentration observed at the same absolute MLSS following training in this study is in line with previous research suggesting that training improves muscle specific clearing capacity of lactate as opposed to reducing production mechanisms. Increased muscle specific clearing capacity of lactate has been shown in both rodents (Donovan, 1983) and humans (MacRae et al., 1992), with increased abundance of the monocarboxylate transport proteins (MCT), which facilitate lactate transport, attributed to this improvement. Whilst MCT expression was not measured in the present study, it seems feasible that the training period increased mechanisms responsible for lactate clearance.

The question as to whether interval or continuous training is more beneficial in stimulating MCT increases has been addressed by a number of research groups, with the culmination of this data suggesting that high intensity interval training is the most efficient method of increasing MCT1 and MCT4 protein content in muscle. Baker et al., (Baker et al., 1998) compared high intensity training (31 $m \cdot min^{-1}$, 15% grade) to moderate intensity training (21 $m \cdot min^{-1}$, 8% grade) over a three-week period in rats. It was shown that high intensity exercise mediated increase of MCT1 protein in soleus and red gastrocnemius whilst there was no effect of moderate intensity exercise on MCT content in similar skeletal muscle sections.

Evertsen et al., (Evertsen, 2001) performed a similar study comparing exercise intensity adaptation, this time in humans. The authors found no change in MCT content following the training period, however did observe that moderate intensity exercise caused a reduction in MCT1, whereas high intensity training

maintained MCT1 content in the muscle. To date, only one study has examined the relationship between lactate transporters and exercise at MLSS. This study found that individual differences in buffering capacity may contribute to the sustainable lactate concentration at MLSS; however there was no obvious correlation observed between MCT1 and MCT4 expression and individual lactate concentrations at MLSS (Lokkegaard, 2001).

The training period increased the running speed at the lactate threshold by 7% in CON and 9% in INT, whilst increasing $\dot{V}O_2$max by 10% and 6% in CON and INT respectively. Alterations in running speed at MLSS, LT and $\dot{V}O_2$max were shown to correlate significantly (P<0.05) before and after the training period suggesting that similar mechanisms govern performance at each marker. Research would suggest that training at an exercise intensity corresponding to a specific endurance parameter (i.e. LT, MLSS) improves performance at this marker (Belman and Gaesser, 1991, Weltman et al., 1992, Carter et al., 1999). However, in a highly trained state there appears predominantly to be an improvement in submaximal endurance capacity, due primarily to achievement of maximal $\dot{V}O_2$max values. Once a plateau in $\dot{V}O_2$max is achieved, submaximal improvement in aerobic performance (i.e. the reduced oxygen cost at LT and MLSS) appears to be the primary factor improving endurance performance (Conley and Krahenbuhl, 1980, Jones, 1998).

Training at the MLSS could therefore be an important addition to moderate and well trained athletes as it appears to provide a strong stimulus for improving both sub-maximal and maximal aspects of aerobic capacity. Further, it is also important to note that the inclusion of two sessions per week of training

corresponding to the MLSS was sufficient to elicit the improvements described post training. Using an inclusion design such as this makes the incorporation of MLSS specific training into an athlete's active program feasible and potentially valuable to induce rapid performance gains at MLSS intensity without substantially disrupting the balance of the training design.

In summary, our data clearly demonstrates the adaptable nature of MLSS to training and how training at a running speed relative to MLSS can improve the physiological responses at LT, MLSS and $\dot{V}O_2$max. It appears that the best approach for an individual to improve MLSS is for them to specifically train at this intensity. The question as to whether short bouts of exercise at intensities exceeding MLSS (i.e. high intensity interval training) would be as beneficial as training at MLSS clearly warrants further investigation, as high intensity interval training has been shown to rapidly increase expression of MCT protein and improve mechanisms of lactate clearance during exercise.

CHAPTER VI – METABOLIC RESPONSES DURING EXERCISE TO EXHAUSTION AT AND ABOVE THE MAXIMAL LACTATE STEADY STATE (MLSS)

6.1. Introduction

Baron and colleagues have previously demonstrated that, in addition to systemic lactate, the MLSS also represents a steady state for some, but not all, physiologically relevant metabolites (Baron et al., 2003, Baron et al., 2008). During 30 min cycling at the MLSS, these authors observed a progressive increase in ammonia, epinephrine and nor-epinephrine coupled with a reduction in systemic pH. This would suggest a dissociation between lactate turnover and other metabolic systems which may be involved in muscle contraction, substrate utilisation and the onset of fatigue at the MLSS (Brooks, 1998).

In a recent review of the MLSS, Billat and co-workers identified three key topics that have yet to be examined (Billat et al., 2003). First, there is limited data regarding physiological responses during exercise to exhaustion at the MLSS (i.e. beyond the standard 30 min exercise protocol). Second, very few studies have examined the physiological responses during exercise at and above the MLSS, and finally, there is no research investigating the causes of fatigue during exercise to exhaustion at and above the MLSS. As a result, much of the information we have regarding the MLSS is drawn from research conducted at exercise intensities close to the MLSS, or based on circulatory lactate concentrations or respiratory parameters, both indirect approaches (Dekerle et al., 2003, Pringle and Jones, 2002, Brickley et al., 2002, Brickley et al., 2007).

Acid base physiology describes the production, regulation and balance of acid exchange between muscle and the circulation (Stewart, 1978, Stewart, 1983). In particular, the concentrations of strong ion electrolytes appear to play pivotal roles in the regulation of muscle function during contraction. The most relevant strong ions with regard to acid base physiology are sodium, potassium, magnesium, chloride, sulphate, lactate, acetoacetate and beta-hydroxy butyrate (Stewart, 1978, Stewart, 1983). Stewart hypothesised that the net effect of the presence of strong electrolyte ions could be expressed in terms of the difference between the total concentration of strong base cations and strong acid anions. Accordingly, the strong ion difference (SID) in a solution is the sum of all strong base cation concentrations, minus all strong acid anion concentrations (Stewart, 1978, Stewart, 1983).

With regard to human acid base balance during exercise, the levels of the major acid of relevance, hydrogen ion, is governed by the strong ion difference, the carbon dioxide partial pressure, and the total weak acid present (Stewart, 1978, Stewart, 1983). Therefore, the concentration of hydrogen ions can be manipulated by alterations in any one of these three parameters. The same regulations also apply for bicarbonate concentrations which buffer and neutralize acid released into the cell. Stewart (1978) also highlights that the membrane transport of these ions and the generation of CO_2 during metabolism can alter the cellular pCO_2 to regulate hydrogen ion exchange. The consequence is that interactions between intracellular ion pools, membrane transporter activity and extracellular ion concentrations ultimately regulate systemic acid base balance.

During exercise, the exchange of strong ions, CO_2 and H_2O between the intracellular and extracellular compartments helps to restore acid–base balance (Lindinger et al., 1992, Lindinger et al., 1994). Lactate produced during exercise is released from the active muscles into the plasma compartment along with potassium (K^+). In contrast, sodium (Na^+) and chloride (Cl^-) are taken into the muscle allowing ion exchange. This ionic flux changes the relative intracellular balance of the anions and cations, which in turn reduces intracellular [H^+] concentrations (Stewart, 1983). In addition, the influx of fluid from plasma into intracellular muscle compartments reduces the concentration of intracellular total weak acids [Atot] thereby altering SID and leading to further increases in plasma [H+] content (Putman et al., 2003).

It is has been clearly demonstrated, by a number of independent research groups (Beneke and von Duvillard, 1996, Billat et al., 2004, Billat et al., 2003, Dekerle et al., 2003, Pringle and Jones, 2002), that when exercise progresses to an intensity exceeding the MLSS there is a sustained elevation in circulating lactate concentrations. What is unknown is whether other metabolites or electrolytes are in equilibrium at MLSS, and whether exercise above MLSS causes a concomitant increase in these metabolites contributing towards the aetiology of metabolic fatigue.

Therefore the present chapter sought to address the following questions:

1. Does the MLSS represent a steady state in acid base balance?
2. Does exercise above the MLSS produce alterations in acid base balance which contribute to fatigue?

It was hypothesised that exercise at and above the MLSS would result in the accumulation of a number of metabolites, independent of lactate, which may contribute to fatigue during exercise.

6.2. Methods

6.2.1. Participants

Eight male, recreationally active volunteers (25 ± 5yr; 179 ± 3.2cm; 77.4 ± 6.2kg mean ± SD) took place in the present study after completing the preliminary testing criteria defined in the general methods section of this thesis.

6.2.2. Experimental trials

Each subject performed two trials to exhaustion at their pre-determined MLSS and two trials to exhaustion at a work intensity 10W > MLSS. Each subject consumed a standard meal (1250kcal: 65% carbohydrate, 21% fat, 14% protein) on the evening prior to the trial and then fasted for 12h, refrained from caffeine, alcohol or strenuous exercise in the 48h prior to exercise. On the morning of the experimental trial, participants awoke and consumed a light snack (320 kcal: 75% carbohydrate, 10% fat, 15% protein) at 07:00 before reporting to the laboratory at 09:00. On arrival at the laboratory, participants rested in a supine position for 30 minutes with heart rate measured by telemetry and respiration and blood measured every five minutes prior to exercise.

Participants completed a standardized warm up on the cycle ergometer for 5 minutes before beginning the experimental trial. The order of each trial was randomised and participants blinded to the exercise intensity and the exercise time. No verbal encouragement was provided during the exercise and exercise

was terminated upon volitional exhaustion. Following exercise, participants returned to the supine position where blood, heart rate and respiratory parameters were measured until each variable returned to baseline conditions. Venous and arterialised bold was collected as previously described in the general methods section of this thesis.

6.2.3. Blood gas and metabolite measurement

Blood gas and metabolite concentrations were measured simultaneously using a GEM Premier 3000 analyser (Instrumentation Laboratories, Warrington, UK). Briefly, each sample was collected in a 10ml syringe, obtained from an antecubital vein and ~1ml of whole blood was analysed immediately for pH, pCO_2, pO_2, Na^+, K^+, Ca^{2+}, Hct, HCO_3^-, TCO_2, Base Excess, SO_{2C}, THbc. Whole blood lactate and glucose concentrations were analysed using a YSI analyser as previously described for capilliarized blood.

6.2.4. Statistical analysis

All results are reported as Mean ± Standard Error of the Mean (SEM) unless otherwise stated. Statistical tests were conducted using the SPSS statistical package. Time to exhaustion during the two exercise trials was analysed via a one-way t-test. Differences in cardio-respiratory and metabolite concentrations during the MLSS and 10W>MLSS trials were evaluated via a two-way ANOVA. Analysis was conducted to measure differences between trials (GROUP) and differences with time (TIME) during exercise. When significance was observed, a tukey's post hoc test was conducted to identify the data point at which the

significant difference had occurred. During all graphical representation, * represents a statistical significance of $P<0.05$.

6.3. Results

Mean parameters of endurance capacity are reported in table 6.1.

Table 6.1. Parameters of endurance capacity (Mean ± SEM)

Variable	Mean ± SEM
Power output at LT (W)	145 ± 14
Power output at MLSS (W)	236 ± 9
Power output at $\dot{V}O_2$max (W)	350 ± 7
$\dot{V}O_2$max ($l \cdot min^{-1}$)	4.1 ± 0.1
HR at $\dot{V}O_2$max ($Beats \cdot min^{-1}$)	192 ± 2

Table 6.2. reports physiological responses during exercise at the LT, MLSS and $\dot{V}O_2$max. There were significant increases in lactate concentrations at the MLSS and $\dot{V}O_2$max in comparison to the LT ($P<0.05$). In contrast, pyruvate concentrations did not change between the LT and MLSS, however they were significantly elevated at $\dot{V}O_2$max compared to LT ($P<0.05$). Accordingly, the lactate to pyruvate ratio was increased at MLSS and $\dot{V}O_2$max compared to the LT ($P<0.05$). There were no other significant differences in physiological responses at the LT compared to the MLSS.

Table 6.2. Metabolic and cardiorespiratory responses at the LT, MLSS and VO_2max (Mean ± SEM; *Difference between LT and MLSS; **Difference between MLSS and VO_2max *P* < 0.05)

Variable	LT	MLSS	$\dot{V}O_2$max
Lactate (mmol·l^{-1})	2.71 ± 0.03	4.84 ± 0.04*	8.1 ± 0.26**
Pyruvate (mmol·l^{-1})	0.33 ± 0.001	0.43 ± 0.001	0.53 ± 0.001*
Lactate to Pyruvate Ratio	8.21 ± 0.07	11.25 ± 0.07*	14.46 ± 0.54**
% $\dot{V}O_2$max	63 ± 2	70 ± 3	---
$\dot{V}O_2$ (l·min^{-1})	2.54 ± 0.04	2.79 ± 0.06	4.01 ± 0.05*
VE (l·min^{-1})	66.4 ± 1.99	73.6 ± 2.08	86.24 ± 1.94*
RER	0.92 ± 0.001	0.96 ± 0.001	1.08 ± 0.004*
Heart Rate (beats·min^{-1})	154 ± 7	165 ± 2	189 ± 3*
RPE	13.7 ± 1.2	14.8 ± 0.25	18.6 ± 0.5*

There was a significant reduction in performance time during exercise 10W>MLSS compared to exercise at the MLSS (49.1 ± 3.2 to 71.2 ± 7.9 min) respectively (*P*<0.05). There was a trend for $\dot{V}O_2, \dot{V}CO_2, \dot{V}E$ and HR to be elevated during exercise 10W>MLSS compared to MLSS, however there were no significant differences in any of the cardio-respiratory variables (Table 6.3).

Table 6.3. Metabolic and cardiorespiratory responses during exercise to exhaustion at and 10W>MLSS. (Mean ± SEM; *P < 0.05)

Variable	MLSS	10W>MLSS
Lactate (mmol·l^{-1})	4.84 ± 0.04	6.42 ± 0.26*
Pyruvate (mmol·l^{-1})	0.43 ± 0.001	0.44 ± 0.001
Lactate to Pyruvate Ratio	11.25 ± 0.07	14.59 ± 0.54*
% $\dot{V}O_2$max	70 ± 1	76 ± 2
$\dot{V}O_2$ (l·min^{-1})	2.79 ± 0.06	3.08 ± 0.05
VE (l·min^{-1})	73.65 ± 2.08	86.24 ± 1.94
RER	0.92 ± 0.001	0.98 ± 0.004
Heart Rate (beats·min^{-1})	164 ± 2	171 ± 3
RPE	14.7 ± 0.2	16.05 ± 0.37

Basal arterialised whole blood lactate concentrations were unchanged prior to exercise in either experimental trial (0.92 ± 0.16 and 1.01 ± 0.14 mmol·l^{-1}; MLSS and 10W>MLSS respectively). In both exercise trials, lactate concentrations were significantly higher than basal conditions for each time point measured. Lactate concentrations increased during the first 5min of exercise at the MLSS (3.27 ± 0.21 mmol·l^{-1} P<0.05), peaked at 4.18 ± 0.31 mmol·l^{-1} after 25min exercise and declined with exercise duration until reaching 3.63 ± 0.26 mmol·l^{-1} at exhaustion (P<0.05). Lactate concentration increased during the first 5min of exercise 10W>MLSS (3.68 ± 0.39 mmol·l^{-1}) and peaked at 5.85 ± 0.39 mmol·l^{-1} at exhaustion (P<0.05) (Figure 6.1). Basal arterialised whole blood glucose concentrations were unchanged prior to exercise (4.37 ± 0.15 and 4.40 ± 0.08 mmol·l^{-1}; MLSS and 10W>MLSS respectively) and did not alter significantly with exercise in either exercise condition. There was a trend for glucose concentrations to decrease during the first 5min of exercise at the MLSS (4.18 ± 0.19 mmol·l^{-1}) and remained suppressed for the entire exercise bout (50min: 4.11 ± 0.27 mmol·l^{-1}). In a similar response, glucose concentrations decreased during the first 5min of exercise above the MLSS (4.26 ± 0.09 mmol·l^{-1}) and

remained suppressed for the entire exercise bout (35min: 4.10 ± 0.23 mmol·l^{-1}). There were no differences in glucose concentrations between exercise at and 10W>MLSS.

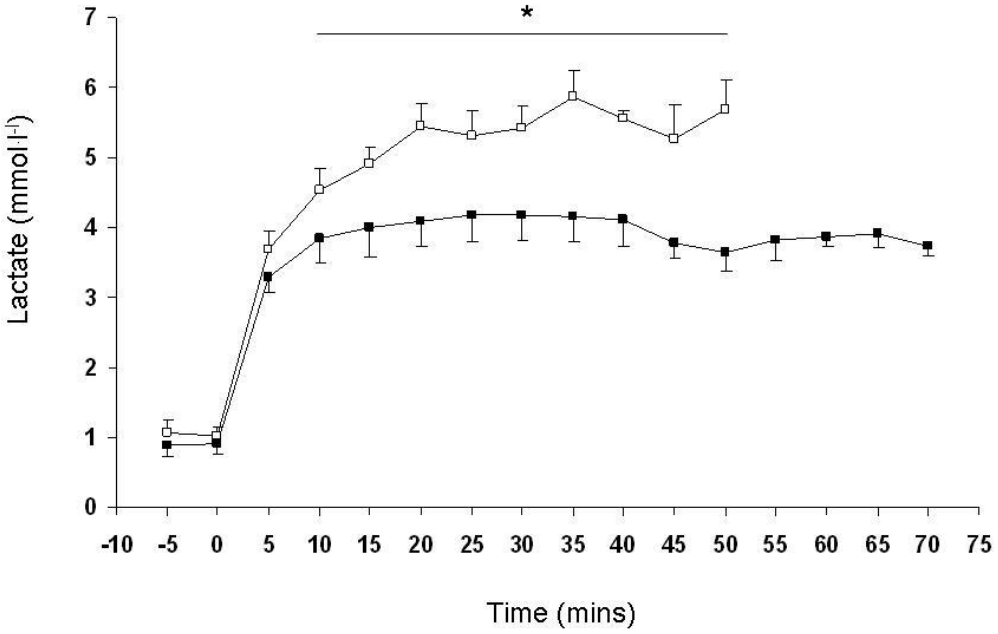

Figure 6.1. Lactate concentrations during exercise to exhaustion at and above the MLSS (* significantly different from MLSS trial *P* < 0.05).

The basal lactate to pyruvate ratio (La:Py) was unchanged prior to exercise in either experimental trial (3.76 ± 0.16 and 2.87 ± 0.16; MLSS and 10W>MLSS respectively). La:Py increased by during the first 5min of exercise at the MLSS (7.60 ± 0.71 *P*<0.05) and continued to increase progressively until peaking at 25min (9.72 ± 0.93 *P*<0.05). La:Py then decreased with continued exercise until termination (50min: 8.40 ± 0.80 *P*<0.05). In contrast, La:Py increased during the first 5min of exercise 10W>MLSS (8.49 ± 0.81 *P*<0.05) and continued to increase during the entire exercise trial, peaking at exhaustion after 35min (13.61 ± 1.31 *P*<0.05).

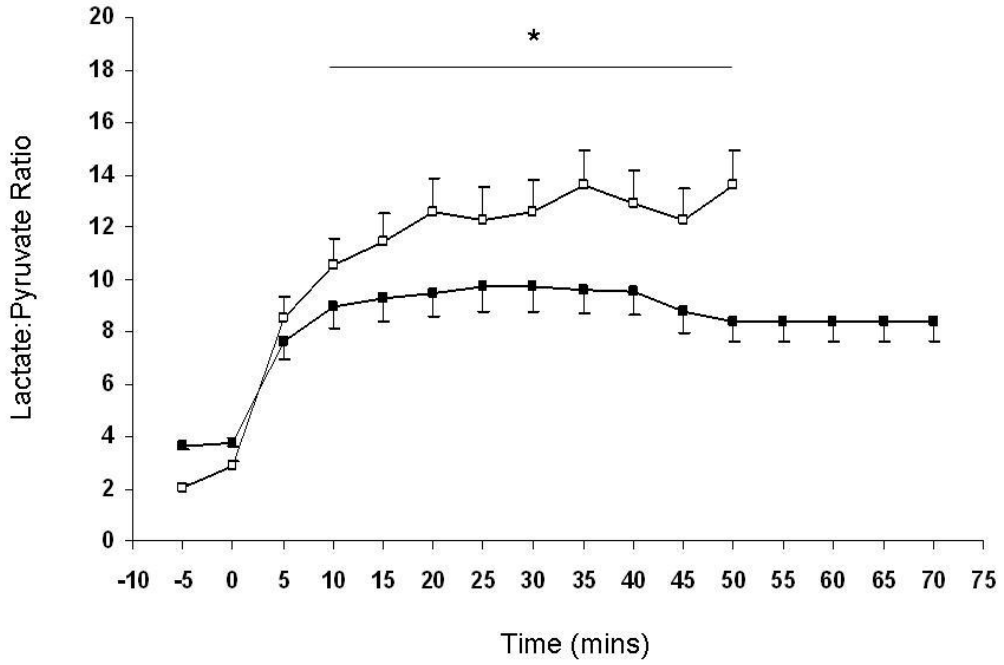

Figure 6.2. Lactate to pyruvate ratio during exercise to exhaustion at and above the MLSS (* significantly different from MLSS trial *P* < 0.05).

There was no difference observed in arterialised blood pH prior to exercise (7.42 ± 0.007 and 7.41 ± 0.005; MLSS and 10W>MLSS respectively). pH decreased by during the first 5min of exercise at the MLSS (7.33 ± 0.01) which was the lowest point recorded. pH gradually returned to basal levels during exercise (50min: 7.40 ± 0.009). Arterialised blood pH decreased during the first 5min of exercise 10W>MLSS (7.34 ± 0.007) remained at this level for the first 25min of exercise (7.35 ± 0.009) and increased slightly upon exhaustion (7.37 ± 0.01).

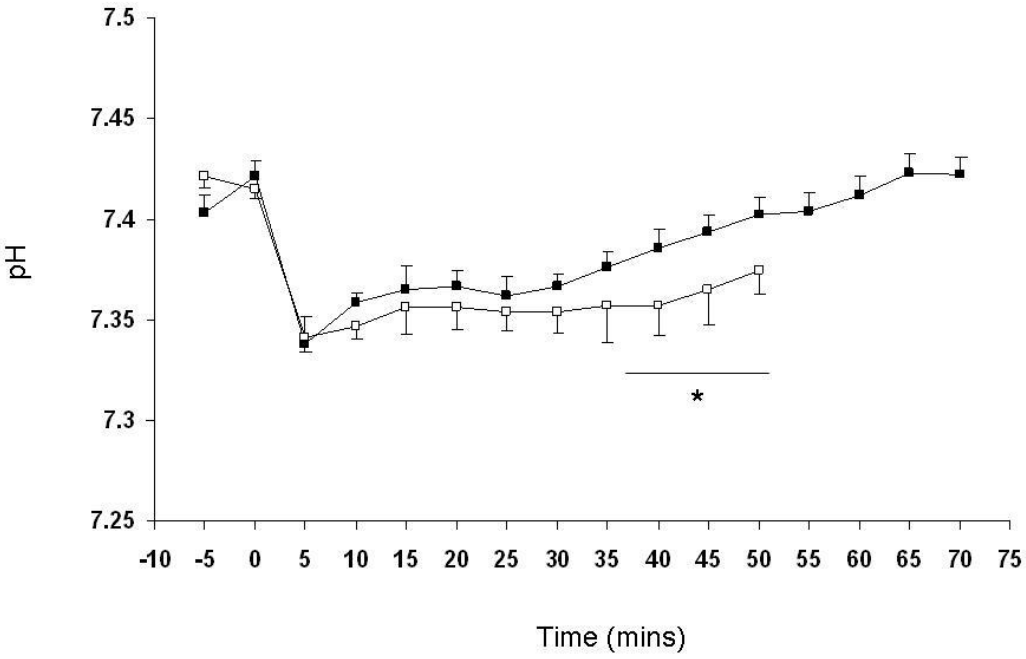

Figure 6.3. Arterialised blood pH during exercise to exhaustion at and above the MLSS (* *P* < 0.05).

A similar response was observed for HCO_3^-. There was no difference observed in arterialised whole blood HCO_3^- prior to exercise (26.85 ± 0.58 and 26.9 ± 0.56 $mmol \cdot l^{-1}$; MLSS and 10W>MLSS respectively). HCO_3^- decreased significantly from basal concentrations in both exercise trials, however there were no significant differences between trials. HCO_3^- decreased during the first 5min of exercise at the MLSS (23.06 ± 0.14 $mmol \cdot l^{-1}$) and reached a nadir after 25min exercise (19.75 ± 0.59 $mmol \cdot l^{-1}$). HCO_3^- then increased gradually during the exercise bout reaching 20.74 ± 0.40 $mmol \cdot l^{-1}$ upon exhaustion. HCO_3^- decreased by during the first 5min of exercise 10W>MLSS (22.81 ± 0.35 $mmol \cdot l^{-1}$) and reached a nadir after 25min exercise (18.74 ± 0.56 $mmol \cdot l^{-1}$). HCO_3^- then increased gradually during the exercise bout reaching 21.37 ± 1.36 $mmol \cdot l^{-1}$ upon exhaustion.

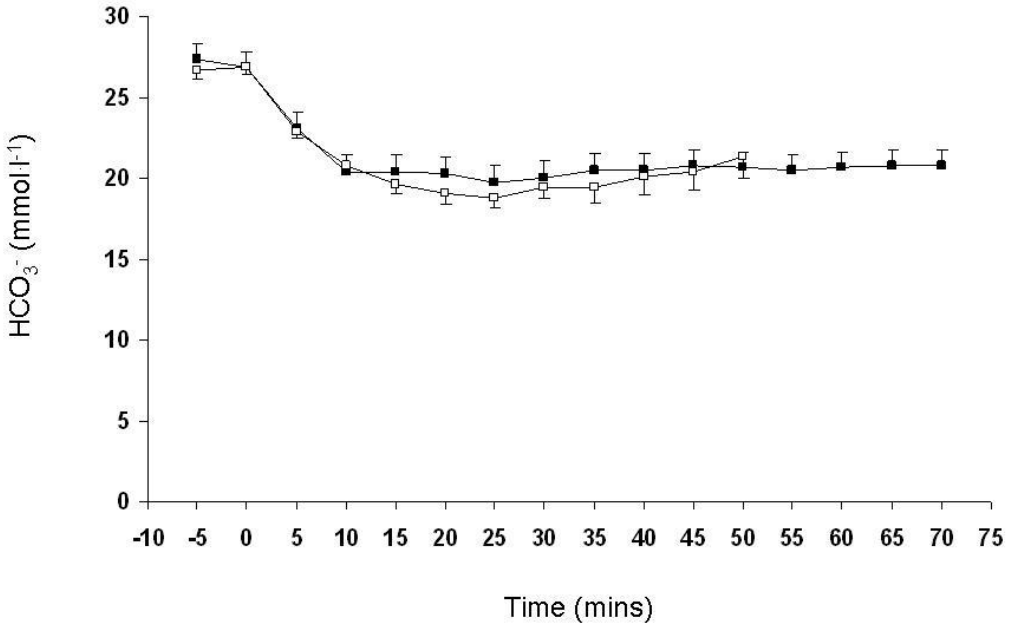

Figure 6.4. Bicarbonate (HCO_3^-) concentrations during exercise to exhaustion at and above the MLSS (* *P* < 0.05).

Base Excess (BE) decreased during both exercise bouts (Figure 6.5). There was no difference observed in basal whole blood BE prior to exercise (2.13 ± 0.40 and 2.06 ± 0.45 mEq/L; MLSS and 10W>MLSS respectively). BE decreased by during the first 5min of exercise at the MLSS (-2.66 ± 0.23 mEq/L) and reached a nadir after 25min exercise (-4.86 ± 0.60 mEq/L). BE then increased gradually during the exercise bout reaching -3.18 ± 0.21 mEq/L upon exhaustion. BE decreased by during the first 5min of exercise 10W> MLSS (-2.9 ± 0.22 mEq/L) and reached a nadir after 25min exercise (-5.88 ± 0.57 mEq/L). BE then increased gradually during the exercise bout reaching 3.37 ± 1.27 mEq/L upon exhaustion.

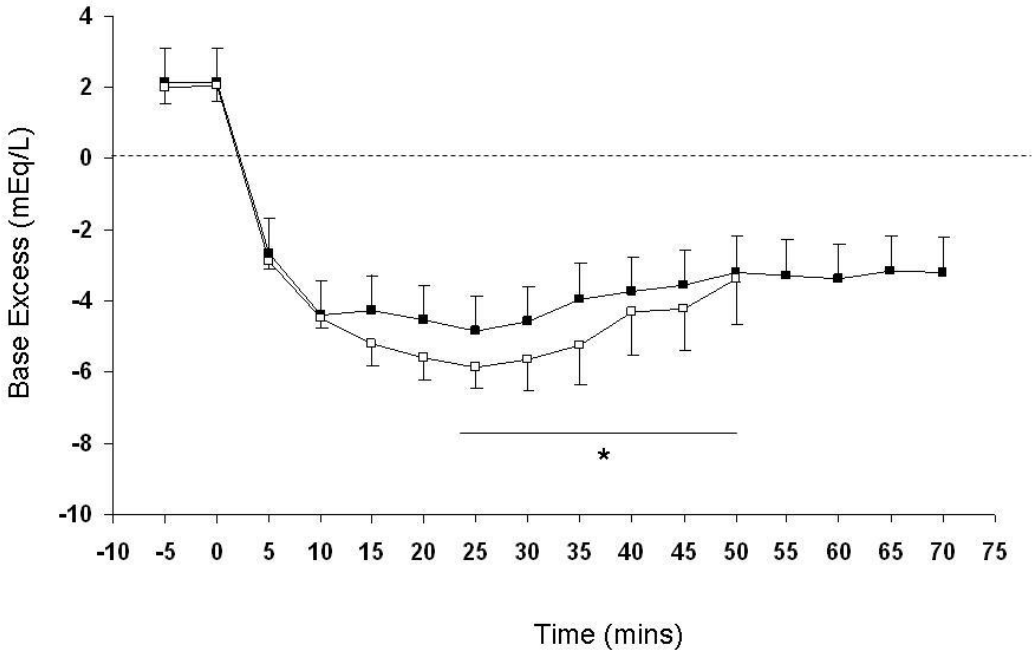

Figure 6.5. Plasma base excess (BE) during exercise to exhaustion at and above the MLSS (* *P* < 0.05).

Basal arterialised sodium (Na^+) concentrations were unchanged prior to exercise (138.6 ± 0.55 and 138.8 ± 0.66 $mmol \cdot l^{-1}$; MLSS and 10W>MLSS respectively). Na^+ increased during the first 5min of exercise at the MLSS (140.8 ± 0.60 $mmol \cdot l^{-1}$) and peaked after 10min exercise (141.8 ± 0.87 $mmol \cdot l^{-1}$). Na^+ declined gradually with exercise duration reaching 139.6 ± 0.46 $mmol \cdot l^{-1}$ upon exhaustion. Na^+ increased during the first 5min of exercise 10W>MLSS (140.6 ± 0.91 $mmol \cdot l^{-1}$) and peaked after 20min of exercise (141.0 ± 0.5 $mmol \cdot l^{-1}$). Na^+ declined gradually with exercise duration reaching 140 ± 1.0 $mmol \cdot l^{-1}$ upon exhaustion.

Basal arterialised potassium (K^+) concentrations were unchanged prior to exercise (4.06 ± 0.13 and 4.11 ± 0.06 $mmol \cdot l^{-1}$; MLSS and 10W>MLSS respectively). K^+ increased during the first 5min of exercise above the MLSS

(4.93 ± 0.17 mmol·l^{-1}) and peaked after 40min exercise (5.3 ± 0.09 mmol·l^{-1}). K^+ remained elevated until exercise exhaustion (5.1± 0.21 mmol·l^{-1}). K^+ increased during the first 5min of exercise 10W>MLSS (5.05 ± 0.05 mmol·l^{-1}) and peaked after 20min exercise (5.46 ± 0.1 mmol·l^{-1}). K^+ remained elevated until exercise exhaustion (4.75 ± 0.34 mmol·l^{-1}).

Arterialised calcium (Ca^{2+}) concentrations were unchanged prior to exercise (1.17 ± 0.008 and 1.15 ± 0.02 mmol·l^{-1}; MLSS and 10W>MLSS respectively). Ca^{2+} increased during the first 5min of exercise at the MLSS (1.215 ± 0.005 mmol·l^{-1}) and returned to basal levels upon exercise exhaustion (1.106 ± 0.02 mmol·l^{-1}). Ca^{2+} increased during the first 5min of exercise 10W>MLSS (1.195 ± 0.01 mmol·l^{-1}) and returned to basal levels upon exercise exhaustion (1.112 ± 0.02 mmol·l^{-1}).

Basal haemoglobin (Hb) concentrations were unchanged prior to exercise (14.5 ± 0.21 and 14.3 ± 0.38 g/dL; MLSS and 10W>MLSS respectively). Hb increased during the first 5min of exercise at the MLSS (15.76 ± 0.24 g/dL), peaked after 10min (15.8 ± 0.17 g/dL) and declined thereafter until exercise exhaustion (15.02 ± 0.13 g/dL). Hb concentration increased during the first 5min of exercise 10W>MLSS (14.98 ± 0.39 g/dL), peaked after 15min (15.14 ± 0.54 g/dL) and decreased below basal levels upon exercise exhaustion (13.65 ± 0.58 g/dL).

Basal heamatocrit (Hct) values were unchanged prior to exercise (42.4 ± 1.05 and 41.33 ± 0.85 %; MLSS and 10W>MLSS respectively). Hct increased during the first 5min of exercise at the MLSS (47.8 ± 0.81 %), peaked after 10min (50 ±

1.46 %) and declined thereafter until exercise exhaustion (46.8 ± 1.04 %). Hct increased during the first 5min of exercise 10W>MLSS (45.66 ± 1.23 %), peaked after 10min (47 ± 1.41 %) and declined thereafter until exercise exhaustion (42 ± 1.11 %).

Basal oxygen saturation (SO_2c) was unchanged prior to exercise (94.8 ± 0.74 and 94.5 ± 0.88 %; MLSS and 10W>MLSS respectively) (Figure 6.6). SO_2c decreased in the first 5min of exercise at the MLSS (92.6 ± 1.02 %), increased above basal conditions after 10 min (95.5 ± 0.56 %) and continued to increase with exercise duration until exhaustion (96.8 ± 0.44 %). SO_2c decreased in the first 5min of exercise at the MLSS (91.1 ± 1.40 %), increased to basal levels after 10min (94.5 ± 0.5 %) and continued to increase with exercise duration until exhaustion (95.7 ± 0.20 %).

Basal oxygen partial pressure (pO_2) was unchanged prior to exercise (90.6 ± 0.56 and 90.28 ± 0.39 mmHg; MLSS and 10W>MLSS respectively). pO_2 decreased in the first 5min of exercise at the MLSS (90.46 ± 0.42 mmHg), recovered during the initial 10min of exercise (100.9 ± 0.40 mmHg) and peaked upon exercise termination (110.6 ± 0.43 mmHg). pO_2 decreased in the first 5min of exercise 10W>MLSS (80.96 ± 0.51 mmHg) and then followed a similar trend to the MLSS trial, increasing above basal conditions after 10min (100.3 ± 0.33 mmHg) and peaking upon exercise termination (100.9 ± 0.29 mmHg).

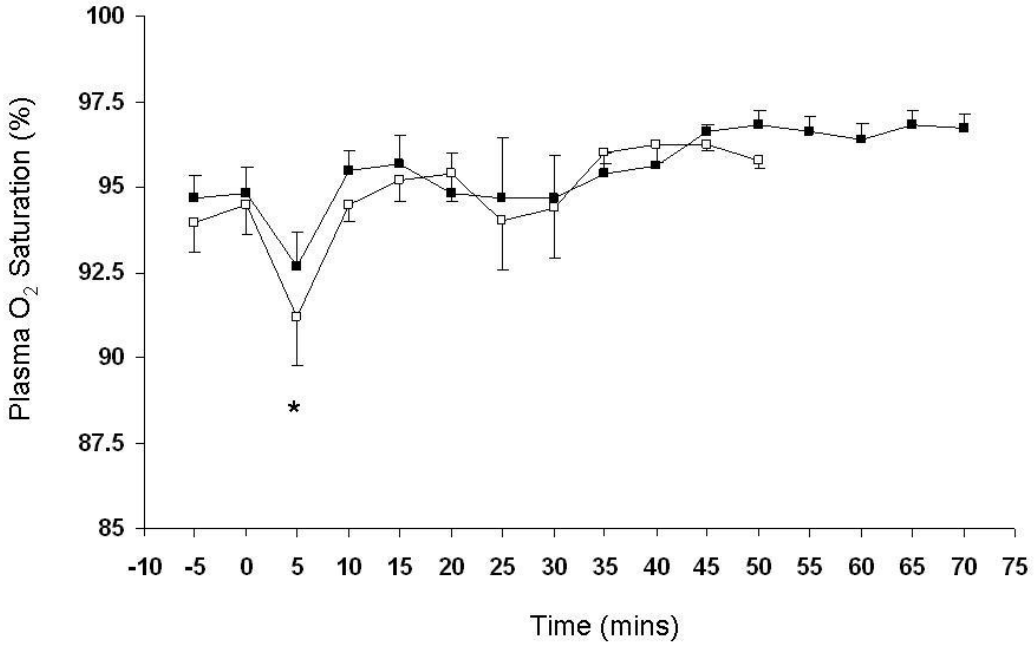

Figure 6.6. Arterialised whole blood oxygen saturation (sO2c) during exercise to exhaustion at and above the MLSS (* *P* < 0.05).

Basal carbon dioxide partial pressure (pCO_2) was unchanged prior to exercise (50.5 ± 1.09 and 50.6 ± 1.04 mmHg; MLSS and 10W>MLSS respectively). pCO_2 increased in the first 5min of exercise at the MLSS, peaking at 50.73 ± 1.90 mmHg. The decrease in pCO_2 continued with exercise duration, reaching a nadir at exercise termination (40.46 ± 1.50 mmHg). pCO_2 was unchanged in the first 5min of exercise 10W>MLSS (50.61 ± 1.60 mmHg), decreased below basal concentrations after 10min (50.05 ± 1.10 mmHg), reached a nadir after 35min exercise before increasing gradually with exercise duration until exhaustion (40.85 ± 2.80 mmHg).

6.4. Discussion

The principal finding from this chapter was that compared to exercise to exhaustion at the MLSS, exercise 10W>MLSS resulted in a significant decrease in exercise performance time, significant increases in lactate and the lactate to pyruvate ratio, without significant alterations in plasma glucose, K^+, Na^+ or Ca^{2+} concentrations. Coupled to these systemic changes was a significant reduction in pH and base excess between the MLSS and >10W MLSS trials. Further, SO_2c, PO_2, Haemoglobin and haematocrit were each significantly reduced during exercise 10W>MLSS compared to MLSS. Collectively this data suggests that the MLSS represents a physiological threshold above which a number of systems involved in cellular homeostasis are compromised. Given that lactate is not the only metabolite altered with the increase in exercise intensity, the present data would support recent suggestions that the aetiology of fatigue during submaximal exercise is more complex than just one single metabolic mechanism (Miller, 2008).

This is the first investigation to compare exercise time to exhaustion at and above the MLSS. Time to exhaustion was reduced by 45% in the 10W>MLSS trial from 71.2 ± 7.9 min at MLSS to 49.1 ± 3.2 min 10W>MLSS respectively. The present study is in agreement with previous studies that have shown that exercise can be maintained at the MLSS for ~60mins (Baron et al., 2008, Fontana et al., 2009, Lajoie et al., 2000). In contrast to the present study, Fontana et al (2009) reported a large variation in individual time to exhaustion at the MLSS (from 30.0 to 65.3 min). Their subject cohort was comprised of moderately trained men, and so differences in individual endurance capacity across their subject population may account for the variation, certainly, the

mean lactate concentrations at MLSS reported by Fontana et al (2009) were higher, and showed greater variance than in the present study. This could suggest that the participants may have had a greater percentage of glycolytic fibres, therefore explaining the increased lactate production at the MLSS (Billat et al., 2003).

6.4.1. Lactate, glucose and pyruvate interactions during exercise

There were no differences in the lactate response during the initial 5 min exercise between exercise intensities, however lactate concentration was significantly higher after ten minutes in the 10W>MLSS trial. Lactate concentrations remained stable and then declined during exercise at the MLSS, at exhaustion the lactate concentration at the MLSS was declining in relation to the first 30min of exercise. The dissociation between lactate concentration and exercise exhaustion is in accordance with Baron et al (2008) who also observed a decrease in systemic lactate concentrations at the termination of exercise at MLSS. The data from the present study and that of Baron et al (2008) certainly question the involvement of lactate in the aetiology of fatigue at the MLSS as originally postulated by Beneke (Beneke, 1995). During exercise above the MLSS, there was a progressive increase in lactate observed, which peaked upon the termination of exercise. Whilst the initial rate of lactate increase appeared to be the same between trials at the onset of exercise (<5 min), lactate concentrations did not stabilize at any point during exercise above the MLSS. In contrast, there were no differences in plasma glucose concentrations observed between trials in either exercise condition.

The lactate to pyruvate ratio followed a similar pattern to the systemic lactate response. Whilst the lactate to pyruvate ratio reached a plateau at the MLSS, there was a progressive increase in this value above the MLSS. This increase was mediated solely by the elevated lactate as there were no differences observed in pyruvate concentrations. This suggests that a maximal rate of pyruvate production had been reached at the MLSS, thus favouring production of lactate via the LDH reaction (Spriet et al., 2000). Explanation for this could be due to one of two mechanisms. Firstly the elevation in glycolytic rate creates a mis-match between glycolytic substrate supply and mitochondrial oxidative capacity, or secondly, substrate saturation of the pyruvate dehydrodgenase complex (PDH) results in an accumulation of pyruvate and a subsequent conversion of pyruvate to lactate (Spriet et al., 2000). The likely scenario is a combination of both of the described mechanisms, however, measuring PDH activity or mitochondrial flux was beyond the scope of the present investigation.

The present study reports similar findings to Poole and colleagues (Poole et al., 1988) who investigated the metabolic and respiratory profile of exercise to exhaustion at and above individual critical power (Poole et al., 1988). Theoretically, the critical power, just as the MLSS, represents the transition from the heavy to severe exercise domains. Exercise time to exhaustion in the study of Poole et al., was, however, significantly shorter than the present study, and the power output at CP significantly higher than the MLSS. Poole et al (1988) reported significant elevations in lactate and the lactate to pyruvate ratio above Critical Power, coupled with a significant reduction in pH, $NaHCO_3$ and PCO_2. Whilst the power output at the critical power does not correspond specifically to that of the MLSS, the present study and Poole et al (1988) suggest that

exercise exceeding the heavy exercise domain, whether it is assessed by the MLSS or critical power results in multifunctional disregulation of processes of lactate production, removal and buffering, resulting in systemic acidosis.

6.4.2. Potassium, sodium and calcium balance

In addition to the increase in systemic lactate, plasma potassium was also observed to increase at both of the exercise intensities examined. However, there was no difference in the potassium response when comparing the MLSS trial to the 10W>MLSS trial. This data is in accordance with previous reports (Lindinger, 1995, Lindinger et al., 1995, Lindinger et al., 1994, Cairns and Lindinger, 2008, McKenna and Hargreaves, 2008) suggesting that potassium efflux from muscle increases with the onset of exercise. Whilst there were no differences in potassium release between the exercise trials, without knowledge of the muscle and interstitial fluid potassium balance, it is impossible to fully understand the role of potassium in the aetiology of fatigue at the MLSS (McKenna and Hargreaves, 2008). The unresponsiveness of plasma Na^+ and Ca^{2+} to exercise is also in agreement with previous reports of Lindinger and colleagues (Lindinger, 1995, Lindinger et al., 1992, Lindinger et al., 1995, Lindinger et al., 1994) and Putman et al (Putman et al., 2003). The increase in systemic lactate and potassium concentrations, without a parallel increase in Na^+ and Ca^{2+} would further support the hypothesis that exercise above the MLSS results in alterations in SID, with lactate, potassium and hydrogen all increased at this exercise intensity.

6.4.3. Exercise 10W>MLSS affects pH balance and buffering capacity

Intense muscle contraction results in the accumulation of lactate and hydrogen ions, causing acidification of the exercised muscle (Brooks, 1999). Acidification has been suggested to be a direct cause of exercise induced muscle fatigue; however this still remains contentious, despite over a century of investigation (Spriet et al., 2008). Skeletal muscle has a number of strategies to buffer hydrogen ion accumulation, thereby maintaining the pH balance of the cell and sustaining exercise. A principal mammalian systemic buffer is bicarbonate (HCO_3^-), which scavenges H^+ to form H_2CO_3. H_2CO_3 then dissociates to CO_2 and H_2O, allowing CO_2 to be removed by the circulatory and respiratory systems (Spriet et al., 2008).

In this study, one of the most obvious differences in the metabolic response at the two exercise intensities studied was the prolonged reduction in pH during exercise at the MLSS, which was significantly lower than corresponding time points at the MLSS. As pH is a net measure of acidity in the blood, the mechanism for this response is likely a combination of increased ion concentrations and/or a decrease in intracellular buffering mechanisms, resulting in a net increase in acidity (Brooks, 1999). Acidosis has long been known to produce profound effects on metabolic function during exercise. Jones and colleagues demonstrated that acidosis could suppress plasma glycerol and free fatty acids during incremental exercise however these changes were independent of cardiovascular changes (Jones et al., 1977).. Starritt and colleagues (Starritt et al., 2000) added further to this observation by demonstrating that a physiological reduction in pH can inhibit CPT1 activity *in*

vitro and therefore blunt fatty acid entry into the mitochondria, adding a possible mechanism to the *in vivo* data of Jones et al, (1977).

More recently, Hollidge-Horvat et al (1999) showed that acidosis resulted in a reduction in intramuscular lactate due to inhibition of glycogenolysis, resulting in reduced pyruvate formation. This reduction appeared to be mediated by acidic suppression on phosphorylase and phosphofructokinase. The authors suggested that reductions in free inorganic phosphate and elevated AMP may have reduced phosphorylase enzyme activity, whilst disregulation in pyruvate metabolism was due to reduced PDH activity, either via allosteric feedback or increased phosphorylation (Hollidge-Horvat et al., 1999). Finally, Jubrias and colleagues, using ^{31}P magnetic resonance (MR) spectroscopy during isometric contraction, demonstrated that acidosis inhibited ATP supply in exercising muscle, resulting in reduced oxidative phosphorylation. Importantly, the negative effects of acidosis were reversed by performing exercise in burst periods, which was shown to increase the ADP:AMP ratio (Jubrias et al., 2003). Unfortunately, the exercise model used by Jubrias et al (isometric contractions in a small muscle group) makes the translation of their results to whole body exercise difficult. The authors argue, that although the oxidative capacity of mitochondria far exceeds the O_2 attained at VO_{2max}, cycling exercise only utilises a small relative muscle mass (Hoppeler et al., 1985, Rowell, 1993) and so it is feasible that intracellular mechanisms may determine oxidative flux in this scenario (Jubrias et al., 2003).

As previously stated, bicarbonate is the principle systemic buffer to negate the exercise induced metabolic acidosis (Spriet et al., 2008). There was a

significant reduction in systemic HCO_3^- in both exercise trials, however there were no differences between trials. Given that HCO_3^- is the principal buffer to counteract metabolic acidosis, it could be that H^+ production >MLSS exceeded the buffering capacity of HCO_3^-, thus resulting in a net increase in lactate concentrations. However, it should also be noted that the release of lactate and hydrogen ions in skeletal muscle have been suggested to occur via independent mechanisms (Bangsbo et al., 1997), therefore potentially dissociating lactate from the intracellular fall in pH observed.

Based on the premise that muscle-buffering capacity is a rate-limiting factor for endurance performance, the hypothesis that alleviating systemic acidosis would promote performance during exercise has been tested for over 75 years. Dennig and colleagues (Dennig et al., 1931) were the first to demonstrate that ingestion of sodium bicarbonate ($NaHCO_3$) prior to exercise could improve running performance. Margaria *et al*., provided further support for the positive role of $NaHCO_3$ in acute endurance performance (Margaria et al., 1971), however research in the following 75 years has failed to fully validate the efficacy of $NaHCO_3$ as an ergogenic aid (Spriet et al., 2008).

$NaHCO_3$ administration during endurance exercise at 60% VO_2max appears to promote glycogenolysis to a level that exceeds the capacity of pyruvate dehydrogenase (PDH) resulting in increased muscle pyruvate and lactate accumulation (Hollidge-Horvat et al., 2000). However, the metabolic shift that occurs within the working muscle has little effect on either pH or fatigue when rodent muscles are electrically stimulated *in vitro* (Broch-Lips et al., 2007). This would suggest that the ergogenic effect of $NaHCO_3$ administration is not due to

decreasing muscle lactate, buffering cytosolic pH, or directly decreasing fatigue, pointing to alternative actions of $NaHCO_3$ and suggesting that NaHCO3 administration might not be an effective tool for improving performance at and above the MLSS.

Whilst measuring the relative HCO_3^- to lactate ratio provides an estimation of buffer capacity, on its own it is a relatively indirect measurement, as bicarbonate concentration is calculated from changes in PCO_2 relative to pH. Astrup and Siggaard-Andersen (Astrup et al., 1960, Astrup and Siggard-Andersen, 1963) were the first to introduce the term Base Excess (BE), in an attempt to add a quantitative measure for the metabolic component of acid-base disturbances (Astrup et al., 1960, Astrup and Siggard-Andersen, 1963). BE is the amount of an acid or alkali required to return pH to a basal metabolic range (Astrup et al., 1960, Astrup and Siggard-Andersen, 1963). Therefore, during clinical stress, or exercise scenarios in which there is prolonged metabolic acidosis, the BE is negative, and indicates the required alkalosis to return pH to basal concentrations (Boning et al., 2007).

BE was significantly lower during exercise 10W>MLSS compared to exercise at the MLSS, supporting the hypothesis that there was a mismatch in ion production and buffering capacities during exercise at this intensity. Unfortunately, it is impossible to dissociate each ionic variable to determine which, if any is detrimental to exercise performance. Currently, the individual importance of lactate and hydrogen ions in the aetiology of fatigue is unresolved. Bangsbo and colleagues (Bangsbo et al., 1997) have previously reported that a dissociation between lactate and hydrogen appearance in

muscle following intense exercise in humans. This led them (Bangsbo et al., 1997), and others (Lindinger and Heigenhauser, 2008) to suggest that H^+ ions compromise muscle function whereas lactate ions, dissociate from hydrogen ions and perform other, positive cellular functions. This hypothesis has however received substantial opposition (Boning and Maassen, 2008) and will remain un-resolved until more advanced analytical tools are available to dissociate the two ions.

In addition to the metabolic parameters previously discussed, exercise above the MLSS also resulted in a significant reduction in oxygen saturation at the onset of exercise, coupled with elevated VO_2 and VE. It should however be noted that after the initial ten minutes of exercise, SO_2c returned to the values reported during exercise at the MLSS. Whilst this deficit at the onset of exercise might have subsequent effects on metabolic responses, the observation that the reduced oxygen saturation was transient, and recovered before exercise termination suggests that this mechanism was not involved in the onset of fatigue.

In summary, this is the first study to investigate the metabolic and respiratory profile of exercise to exhaustion at and 10W>MLSS. The present data adds to previous research dissociating lactate accumulation from fatigue at the MLSS (Baron et al., 2008, Green et al., 2005). In addition, exercise above the MLSS results in a progressive metabolic acidosis, which appeared to be as a result of a co-ordinated increase in lactate, potassium and hydrogen ions, in combination with suppression in HCO_3^- and PCO_2. This observation would therefore suggest that the MLSS also represents the upper limit for systemic buffering capacity

and is an exercise intensity at which multiple ionic interactions contribute to fatigue (Cairns and Lindinger, 2008).

CHAPTER VII – LACTATE AND GLUCOSE KINETICS DURING EXERCISE TO EXHAUSTION AT AND ABOVE THE MAXIMAL LACTATE STEADY STATE (MLSS).

7.1. Introduction

Whilst the practical importance of the power output or work intensity corresponding to the MLSS is well documented (Billat et al., 2003, Jones and Carter, 2000), there is limited information regarding (1) the physiological mechanisms regulating exercise at the MLSS and (2) the physiological mechanisms that contribute to fatigue at intensities above the MLSS. As demonstrated in chapter three of this thesis, there are a number of factors which appear to contribute to the etiology of fatigue above the MLSS which are related to lactate (pyruvate, pH, HCO_3^-) and independent of lactate (oxygen delivery, sodium/calcium/potassium concentrations, balance of substrate utilization).

Fundamentally, the MLSS represents the highest power output or work intensity at which there is balance between the rate of lactate production and the rate of lactate removal, resulting in a net steady state in systemic lactate concentrations (Billat et al., 2003). In order for there to be an increase in systemic lactate concentrations above MLSS, an imbalance must occur between processes of production and removal (Brooks, 1998, Brooks, 2000). However, to date, this process has not been examined in either rodent or human exercise models. Whilst lactate is easily measured in the circulation, a limitation of blood sampling is it represents a net end-point measurement. In reality, the systemic lactate concentration represents the combined total of

substrate energy balance (glucose and lipid utilization), the balance of ion production and buffering capacity of the cell, the co-ordinated ability of lactate transporter proteins to facilitate lactate movement between sites of production and removal, or the capacity of skeletal muscle/liver to utilize lactate. The use of stable isotopes to label substrate or metabolite cellular pools has greatly advanced our understanding of the processes of lactate metabolism during exercise, allowing quantification of the relative contributions of production and removal to net lactate balance (Wolfe, 2005).

Hurley and coworkers (Hurley et al., 1984) were amongst the first to demonstrate in humans that lactate concentrations at the same relative exercise intensities (55-75% of $\dot{V}O_2$max) were significantly lower after training. A blood lactate of 2.5 mmol·l^{-1} was attained at 68 ± 4% $\dot{V}O_2$max before and 75 ± 3% of $\dot{V}O_2$max after training. This suggested that training could reduce the processes of lactate production and increase the lactate clearance ability of skeletal muscle, heart, lungs and liver. Previously, Donovan and Brooks (1983) had demonstrated that lactate clearance capacity increased following training in rat skeletal muscle. Accordingly, increased clearance capacity was the principal reason suggested for the attenuated lactate concentrations post training as depicted by Hurley et al., (1984), however at this point there was no direct evidence available in humans to verify this hypothesis.

The Noakes lab (MacRae et al., 1992) were the first to attempt to directly measure the contribution of the rate of lactate appearance (R_a), rate of lactate disappearance (R_d) and lactate metabolic clearing rate (MCR) at sub-maximal exercise intensities following endurance training. To make these

measurements, MacRae et al., (1992) used radioisotope dilution measurements of L-[U-^{14}C] lactate during exercise. They demonstrated that the slower rise in blood lactate with increasing O_2 uptake ($\dot{V}O_2$) after training was due to a reduced lactate R_a at the lower work rates ($\dot{V}O_2$ less than 60% $\dot{V}O_2$max). In contrast, at less than 75% $\dot{V}O_2$max, peak lactate MCR values were higher after than before training (40 ± 3 vs. 31 ± 4 ml·min^{-1}·kg^{-1}). Thus the lower blood lactate values during exercise after training appeared to be a result of a diminished lactate R_a at low absolute and relative work rates and an elevated MCR at higher absolute and all relative work rates during exercise following endurance training.

The data presented by MacRae et al., (1992) was therefore in contrast to the rodent data presented by the Brooks lab and suggested, contrary to previous hypotheses that the processes involved in lactate generation or appearance during exercise were most impacted upon by endurance training in humans. The methods used by MacRae et al., (1992) were questioned (Brooks, 1991, Brooks, 1998, Brooks, 1999), and were predominantly responsible for the studies performed by Bergman et al., (2000) which used a non-radioactive, stable isotope infusion approach (3-^{13}C lactate and 6, 6-^{2}H glucose), with limb lactate kinetics to comprehensively assess the relative contributions of local and systemic factors in lactate balance during exercise. Consistent with earlier studies, post training total lactate concentrations decreased at the same absolute (ABT) but not at relative (RLT) exercise intensities. Compared with the 65% $\dot{V}O_2$max pre-training condition (4.75 ± 0.4 mmol·l^{-1}), lactate R_a decreased at absolute (41%) and relative (21%) intensities. Further, leg lactate uptake and oxidation as well as MCR were unchanged at ABT but increased at RLT.

Therefore, the data from Bergman et al., (2000) suggests that firstly, active skeletal muscle is not solely responsible for increased lactate appearance during exercise. Secondly, training has a combined effect on lactate kinetics by increasing leg lactate clearance, decreasing whole body and leg lactate production at moderate exercise intensities, and thirdly, training increases both whole body and leg lactate clearance at higher relative power outputs. In a subsequent study, Dubouchard and colleagues (Dubouchaud et al., 2000) demonstrated that the increased leg lactate clearance appeared to be mediated by increased skeletal muscle protein content of MCT1 and LDH, therefore increasing lactate uptake and oxidative processes.

Despite the suggested importance of the MLSS for endurance exercise capacity (Billat et al., 2003), there is no data available examining lactate and glucose kinetics during exercise at the MLSS. It was therefore the aim of the present study to (1) quantify whole body lactate and glucose kinetics during exercise at the MLSS, and (2) examine the processes of increased lactate appearance during exercise above the MLSS. Based on these studies, it was hypothesized that the increased systemic lactate concentrations during exercise above the MLSS would be due to a disproportionate increase in lactate production (R_a) compared to removal (R_d) and clearance (MCR and R_{ox}) capacities.

7.2. Methods

Eight healthy, recreationally active male subjects (mean ± SD: 26 ± 4yr; 176 ± 6.4cm; 75.2 ± 5.4kg) took part in the present study after completing the preliminary testing criteria defined in the general methods section of this thesis.

7.2.1. Experimental trials

Each subject performed four trials to exhaustion, two at their pre-determined MLSS and two trials to exhaustion at a work intensity 10W>MLSS. Each subject consumed a standard meal (1250kcal: 65% carbohydrate, 21% fat, 14% protein) on the evening prior to the trial and then fasted for 12h, refraining from caffeine, alcohol or strenuous exercise in the 48h prior to exercise. On the morning of the experimental trial, participants awoke and consumed a light meal (320 kcal: 75% carbohydrate, 10% fat, 15% protein) at 07:00 before reporting to the laboratory at 09:00. On arrival to the laboratory, participants rested in a supine position for 60 minutes. Background blood and breath samples were collected following the insertion of an 18 gauge cannula in the anticubital vein of the left and right arm. Participants then received either a primed continuous infusion of [6,6-^{2}H]glucose and [3-^{13}C]lactate or [1-^{13}C]Bicarbonate while resting semisupine for 30min. Participants completed a standardized warm up on the cycle ergometer (60RPM for 5 minutes) before beginning the experimental trial. Participants competed two trials at the MLSS (with each tracer condition) and two trials 10W>MLSS (with each tracer condition). The order of each trial was randomized and the participants blinded to the exercise intensity and the exercise time. No verbal encouragement was provided during the experimental trial with exercise terminated upon the attainment of volitional exhaustion.

The priming bolus was equal to 23 times the resting lactate infusion rate (Bergman et al., 1999). Tracer lactate was infused via an Intelligent pump 522 (Kendall McGaw, Irvine, CA) at 2.5 mg/min at rest, 7.5 mg/min during exercise at the MLSS and 10mg/min during exercise above the MLSS. Increases in tracer infusion were designed to elicit similar arterialised enrichments between

exercise intensities during the anticipated steady state period (i.e. minute 10 to exhaustion). Isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA), diluted in 0.9% sterile saline, and tested for sterility and pyrogenicity before use (Eastbourne District General Hospital, Eastbourne, UK). Following completion of exercise, participants returned to the supine position where blood, heart rate and respiratory parameters were determined until each variable returned to baseline conditions.

Blood samples were collected, stored and analyzed as previously described in the chapter three. Pyruvate analysis was conducted as previously described (Bergman et al., 1999).

7.2.2. Plasma [3-13C] lactate analysis

Samples of arterialised blood were analysed for lactate isotopic enrichment via gas chromatography- mass spectrometry (GCMS; GC model 5890 series II and MS model 5989A, Hewlett-Packard) using the TMS-oxime deritive approach, adapted from Tserng and colleagues (Tserng et al., 1984). In preparation for GCMS analysis, samples were deproteinised with cold acetone, centrifuged at 5000rpm and the supernatant transferred to eppendorf vials. The samples were then lyophilized and dissolved in a 100ul solution of hydroxylamine in pyridine (25mg/ml). The samples were incubated at 70°C for 45min and then 100ul of N,O-bis trifluoroacetamide (BSTFA) was added and re-incubated for a further 45min. The derivatized lactate was then analyzed by GCMS. For GCMS analyses, a Perkin Elmer Clarus 500 was used, the injector temperature was set at 240°C; the initial oven temperature was set at 90°C for 5 minutes after injection, then increased to 260°C at 15 C/min, the transfer line was set at

240°C, the source temperature at 180°C. Typically, samples gave sufficient signal size with 0.5 to 1ul injected in this mode. The carrier gas was helium, and split injection of 1:50 was used whilst flow was kept constant. Selective ion monitoring was used to monitor ion mass-to-charge ratios of 117.2 and 118.2 for [^{12}C]- and [^{13}C]lactate, respectively. Breath samples for $^{13}CO_2$ enrichments were collected in glass vacutainers and determined by isotope ratio mass spectroscopy (Metabolic Solutions; Merrimack, NH) as previously described (Bergman et al., 1999b). A bicarbonate correction value of 0.9 was used for each sample timepoint. To monitor the reliability of GCMS analysis, known samples were routinely assessed throughout the study period. An r value >0.98 was consistently observed. Limits of agreement analysis confirmed the reliability of the analysis over the time period.

Lactate rate of appearance (R_a) and rate of disappearance (R_d), metabolic clearance rate (MCR), and oxidation were calculated by using equations defined by Steele (Steele et al., 1956) and modified for use with stable isotopes (Bergman et al., 1999b).

$$R_a \text{ (mg.kg-1.min-1)} = \frac{F - V[(C_1+C_2)/2][IE_2-IE_1)/(t_2-t_1)]}{[(IE_2 + IE_1)/2]}$$

$$R_d \text{ (mg.kg-1.min-1)} = R_a - V[(C_1 - C_2)/(t_2-t_1)]$$

$$MCR \text{ (mg.kg-1.min-1)} = R_d /[(C_1 + C_2)/2]$$

$$R_{ox} \text{ (mg.kg-1.min-1)} = \frac{R_d /[(VCO_2 + IE\ CO2)(100)]}{[(F(k)/89.08]}$$

Figure 7.1 Lactate and glucose kinetics calculations - Where F represents isotope infusion rate, IE_1 and IE_2 are lactate isotopic enrichments at sampling time points 1 (t_1) and 2 (t_2), respectively; C_1 and C_2 are lactate concentrations at t_1 and t_2, respectively; V is the estimated volume distribution of lactate (180 ml/kg); 89.08 is the molecular weight of lactate; and k is a correction factor for retention of tracer in CO_2 pools (0.65 during rest and 0.90 during exercise). Isotopic enrichments of lactate were corrected for background enrichments from blood samples taken before isotope infusion as described in (Bergman et al., 1999b).

7.2.3. Plasma Deuterium Labelled Glucose D-[6,6$^2H_2$] Analysis

Glucose was derivatized in the same samples prepared for lactate analysis and so both metabolites could be analysed in a single GCMS run. Glucose enrichment was analysed by measuring the m/z 319 and 321 signals. Mass peaks of 319 (unlabelled glucose; tracee) and 321 ($^2H_2$ D-Glucose; tracer) were recorded. An analysis of the infused bolus was also measured for comparison.

7.2.4. Statistical analysis

All results are reported as Mean ± Standard Error of the Mean (SEM) unless otherwise stated. Statistical tests were conducted using the SPSS statistical package. Time to exhaustion during the two exercise trials was analysed via a one-way t-test. Differences in cardio-respiratory and metabolite concentrations during the MLSS and 10W>MLSS trials were evaluated via two-way repeated measures ANOVA. Analysis was conducted to measure differences between trials (GROUP) and differences with time (TIME) of exercise. When significance was observed, a tukey's post hoc test was conducted to identify the group and

time point at which the significant difference occurred. During all graphical representation, * represents a statistical significance of $P<0.05$.

7.3. Results

Mean parameters of endurance capacity are reported in table 7.1.

Table 7.1. Parameters of endurance capacity (Mean ± SEM)

Variable	Mean ± SEM
Power output at LT (W)	151 ± 8
Power output at MLSS (W)	242 ± 10
Power output at $\dot{V}O_2$max (W)	358 ± 9
$\dot{V}O_2$max (l·min^{-1})	4.2 ± 0.8
HR at $\dot{V}O_2$max (Beats·min^{-1})	189 ± 6

Time to exhaustion - There was a significant reduction in performance time during exercise above the MLSS compared to exercise at the MLSS (33.5 ± 2.7 to 48.9 ± 5.9 min) respectively (Figure 7.2).

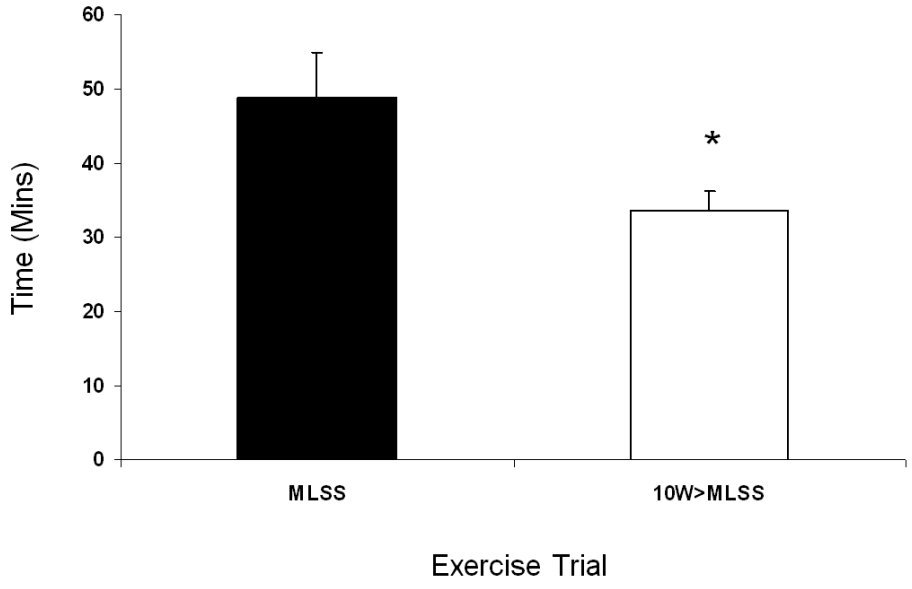

Figure 7.2. Exercise time to exhaustion at and above the MLSS (* P < 0.05)

Cardio-respiratory parameters at and above the MLSS - There was no difference in basal $\dot{V}O_2$ in each experimental trial (0.23 ± 0.03 and 0.26 ± 0.01 l·min^{-1}; MLSS and 10W>MLSS respectively). $\dot{V}O_2$ increased in the first 5min of exercise at MLSS to 2.36 ± 0.18 l·min^{-1}. $\dot{V}O_2$ peaked after 35min (2.81 ± 0.15 l·min^{-1}) and then decreased until exercise was terminated (2.53 ± 0.15 l·min^{-1}). $\dot{V}O_2$ during exercise 10W>MLSS showed a similar trend, however $\dot{V}O_2$ increased at 5min (2.75 ± 0.10 l·min^{-1}) and then continued to increase until peaking at the termination of exercise (3.04 ± 0.13 l·min^{-1}). There was no difference in basal $\dot{V}CO_2$ in each experimental trial (0.20 ± 0.03 and 0.23 ± 0.03 l·min^{-1}; MLSS and 10W>MLSS respectively). $\dot{V}CO_2$ increased in the first 5min of exercise at MLSS to 2.46 ± 0.16 l·min^{-1}. $\dot{V}CO_2$ peaked after 35min (2.70 ± 0.11 l·min^{-1}) and then decreased until exercise was terminated (2.46 ± 0.18 l·min^{-1}). $\dot{V}CO_2$ during exercise 10W>MLSS showed a similar trend, $\dot{V}CO_2$ increased in the first 5min of exercise (2.45 ± 0.37 l·min^{-1}) and then continued to increase until peaking at the termination of exercise (2.91 ± 0.20 l·min^{-1}).

There was no difference in basal RER between experimental trials (0.88 ± 0.07 to 0.87 ± 0.02; MLSS and 10W>MLSS respectively). RER increased during the first 5min of exercise at the MLSS (1.04 ± 0.02) and then decreased with exercise duration to 0.967 ± 0.02 at exhaustion (Figure 4.3). RER 10W>MLSS increased during the first 5min of exercise (1.04 ± 0.02) and in contrast to the MLSS trial remained above 1.00 for the first 15min of exercise (1.00 ± 0.04). RER decreased at exhaustion (0.95 ± 0.04) (Figure 4.3).

There was no difference observed in basal heart rate prior to exercise (62 ± 3 to 67 ± 2 beats·min^{-1}; MLSS and 10W>MLSS respectively). Heart rate at MLSS increased in the first 5min of exercise (148 ± 2 beats·min^{-1}) and continued to increase until peaking at the termination of exercise (181 ± 2 beats·min^{-1}). Heart rate during exercise above the MLSS increased during the first 5min of exercise (152 ± 2 beats·min^{-1}) and continued to increase until peaking at the termination of exercise (179 ± 2 beats·min^{-1}).

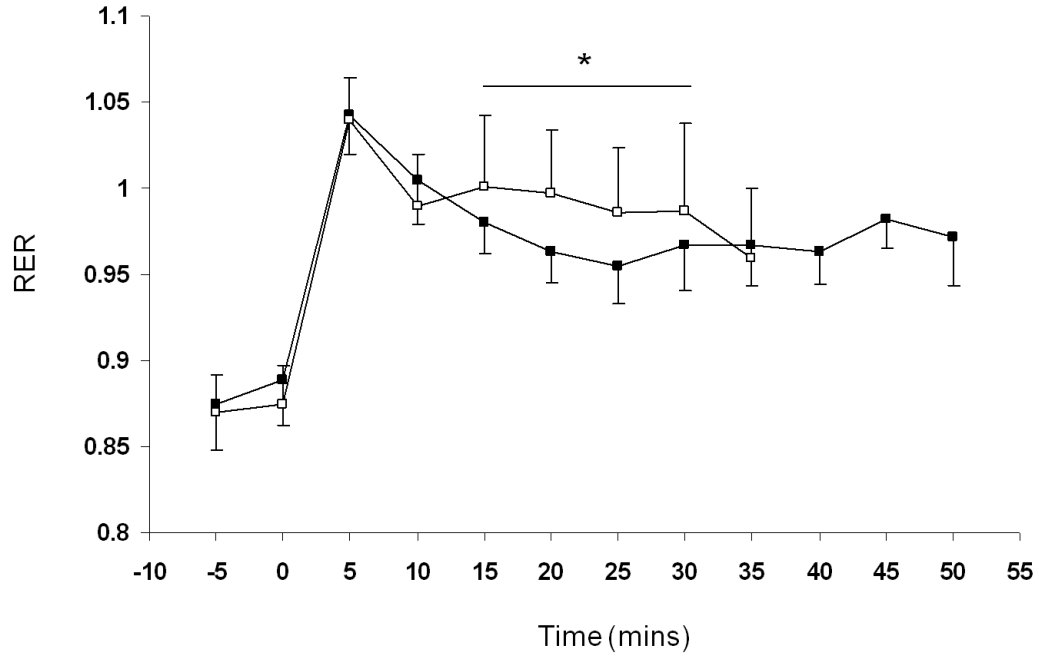

Figure 7.3. RER during exercise to exhaustion at and above the MLSS (* P < 0.05)

There was no difference in basal RPE scores in either exercise trial (6 for both the MLSS and 10W>MLSS trials). RPE increased to 12.2 ± 0.7 during the first 5min of exercise at the MLSS and continued to increase until exercise exhaustion (18.5 ± 1.5). During the 10W>MLSS trial, RPE increased to 12.4 ± 0.8 during the first 5min of exercise and continued to increase until peaking at exhaustion (18 ± 1).

Blood lactate and glucose concentrations – Basal arterialized lactate concentrations were unchanged prior to exercise (0.92 ± 0.16 and 1.01 ± 0.14 mmol l^{-1}; MLSS and 10W>MLSS respectively). Lactate increased during the first 5min of exercise at the MLSS (3.27 ± 0.21 mmol l^{-1}), peaked at 4.18 ± 0.31 mmol l^{-1} after 25min exercise and declined with exercise duration until reaching 3.63 ± 0.26 $m \cdot mol^{-1}$ at exhaustion. Arterialised lactate concentrations increased by during the first 5min of exercise above the MLSS (3.68 ± 0.39 mmol l^{-1}) and peaked at 5.85 ± 0.39 mmol l^{-1}at exhaustion.

Arterialised glucose concentrations were not different prior to exercise (4.37 ± 0.15 and 4.40 ± 0.08 mmol l^{-1}; MLSS and 10W>MLSS respectively). Glucose decreased by during the first 5min of exercise at the MLSS (4.18 ± 0.19 mmol l^{-1}) and remained suppressed for the entire exercise bout (50min: 4.11 ± 0.27 mmol l^{-1}). Whole blood glucose decreased during the first 5min of exercise above the MLSS (4.26 ± 0.09 mmol l^{-1}) and remained suppressed for the entire exercise bout (35min: 4.10 ± 0.23 mmol l^{-1}).

Lactate and glucose kinetics at and above the MLSS - Basal lactate R_a was the same between trials (3.02 ± 0.65 and 2.4 ± 0.23 $mg \cdot kg^{-1} \cdot min^{-1}$; MLSS and 10W>MLSS respectively). R_a increased in the first 5 minutes of exercise at MLSS (8.31 ± 0.71 $mg \cdot kg^{-1} \cdot min^{-1}$) and peaked at 20min of exercise (10.55 ± 0.77 $mg \cdot kg^{-1} \cdot min^{-1}$). R_a continued to decrease with exercise duration until exercise termination at which point R_a was 6.71 ± 1.21 $mg \cdot kg^{-1} \cdot min^{-1}$. In contrast, R_a increased during the first 5 minutes exercise above the MLSS (12.33 ± 1.13 $mg \cdot kg^{-1} \cdot min^{-1}$). R_a peaked after 10 minutes of exercise (12.57 ± 0.68 $mg \cdot kg^{-1} \cdot min^{-1}$) and remained elevated until 20 minutes (12.54 ± 0.97 $mg \cdot kg^{-}$

$^{1.}$min^{-1}). Similarly to the MLSS exercise trial, R_a declined at exhaustion (8.86 ± 2.22 mg·kg$^{-1.}$min^{-1}) (Figure 4.5).

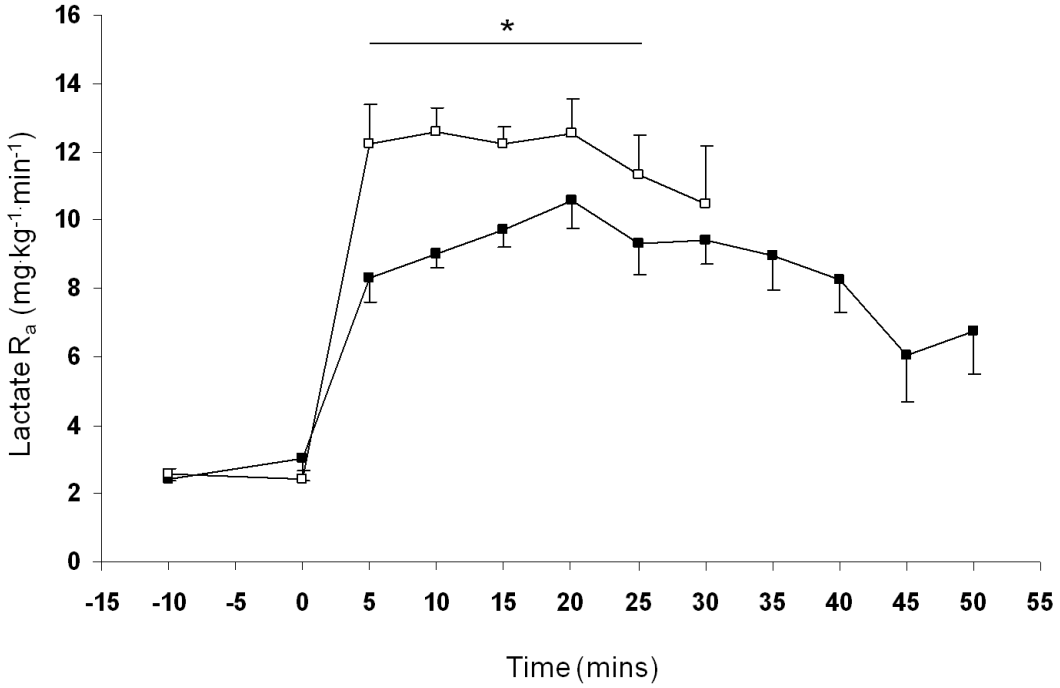

Figure 7.4. Lactate rate of appearance (R_a) during exercise to exhaustion at and above the MLSS (* P < 0.05)

Basal lactate R_d was the same between trials (3.06 ± 0.65 and 2.47 ± 0.24 mg·kg$^{-1.}$min^{-1}; MLSS and 10W>MLSS respectively). R_d increased in the first 5 minutes of exercise at MLSS (3.50 ± 0.97 mg·kg$^{-1.}$min^{-1}) and peaked at 20min of exercise (10.91 ± 0.81 mg·kg$^{-1.}$min^{-1}). R_d continued to decrease with exercise duration until exercise termination at which point R_d was 6.73 ± 1.12 mg·kg$^{-1.}$min^{-1}. In contrast, R_d increased during the first 5 minutes exercise above the MLSS (3.49 ± 1.34 mg·kg$^{-1.}$min^{-1}). R_d peaked after 25min of exercise (11.97 ± 0.66 mg·kg$^{-1.}$min^{-1}) and declined until the termination of exercise (9.55 ± 1.82 mg·kg$^{-1.}$min^{-1}).

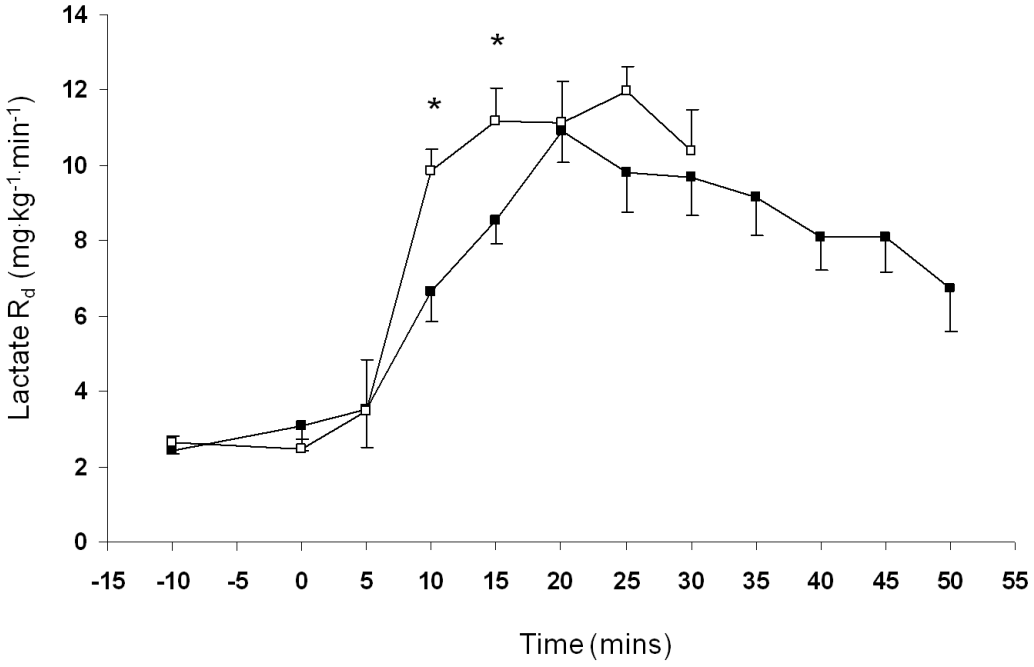

Figure 7.5. Lactate rate of disappearance (R_d) during exercise to exhaustion at and above the MLSS (* P < 0.05)

Basal lactate MCR was the same between trials (4.19 ± 0.47 and 3.43 ± 0.63 mg.kg.min; MLSS and 10W>MLSS respectively). MCR increased in the first 5 minutes of exercise at MLSS (19.53 ± 4.85 mg·kg^{-1}·min^{-1}) and peaked at 20min of exercise (26.5 ± 1.95 mg·kg^{-1}·min^{-1}). MCR continued to decrease with exercise duration until exercise termination at which point MCR was 19.9 ± 2.99 mg·kg^{-1}·min^{-1}. In contrast, MCR increased during the first 5 minutes exercise above the MLSS (19.88 ± 8.32 mg·kg^{-1}·min^{-1}). MCR peaked after 10 minutes of exercise (28.8 ± 3.24 mg·kg^{-1}·min^{-1}) but declined at exhaustion (22.59 ± 4.14 mg·kg^{-1}·min^{-1}).

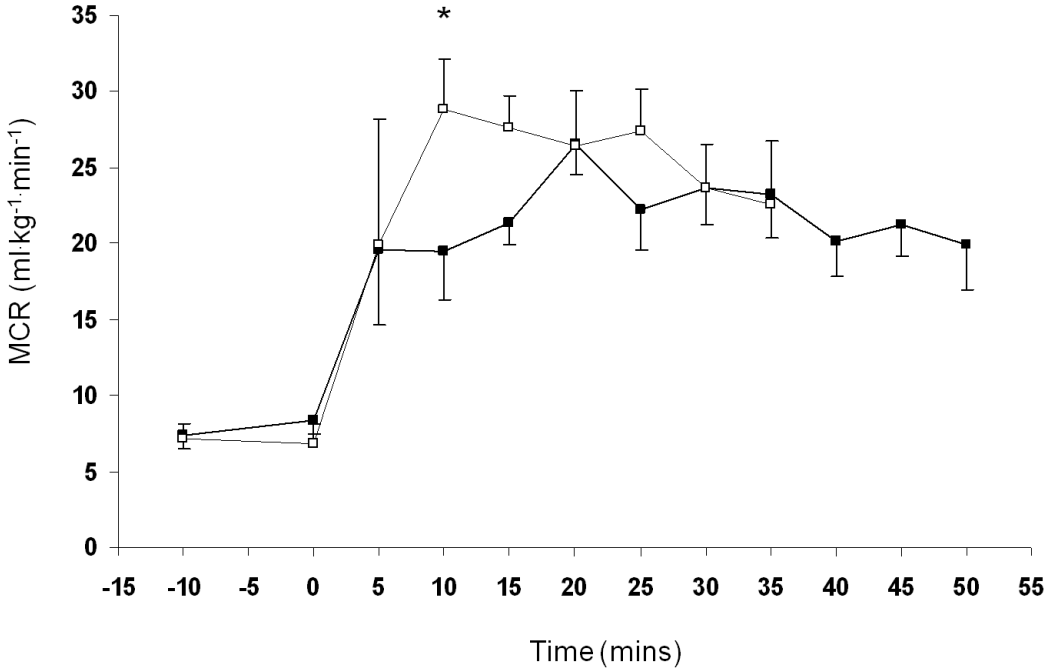

Figure 7.6. Lactate metabolic clearance rate (MCR) during exercise to exhaustion at and above the MLSS (* P < 0.05)

Basal lactate R_{OX} was the same between trials (0.52 ± 0.13 and 0.59 ± 0.06 mg.kg.min; MLSS and 10W>MLSS respectively). R_{OX} increased by during the first 10 minutes of exercise at MLSS (6.50 ± 0.75 $mg \cdot kg^{-1} \cdot min^{-1}$) and peaked at 35min of exercise (6.97 ± 0.72 $mg \cdot kg^{-1} \cdot min^{-1}$). R_{OX} continued to decrease with exercise duration until termination at which point R_{OX} was 6.21 ± 0.51 $mg \cdot kg^{-1} \cdot min^{-1}$. R_{OX} increased during the first 10 minutes exercise 10W>MLSS (7.05 ± 0.27 $mg \cdot kg^{-1} \cdot min^{-1}$). R_{OX} peaked after 30 minutes of exercise (7.60 ± 0.32 $mg \cdot kg^{-1} \cdot min^{-1}$) but declined at exhaustion (7.49 ± 0.48 $mg \cdot kg^{-1} \cdot min^{-1}$).

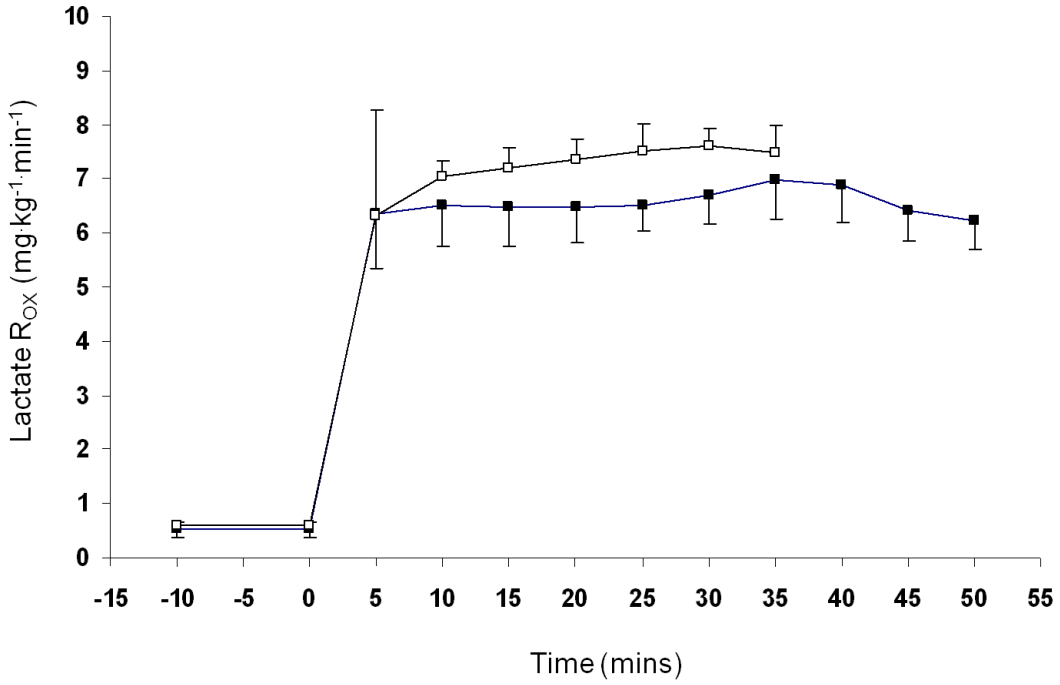

Figure 7.7. Rate of lactate oxidation (R_{OX}) during exercise to exhaustion at and above the MLSS (* P < 0.05)

Basal glucose R_a was the same between trials (3.36 ± 0.70 and 2.11 ± 0.24 mg·kg^{-1}·min^{-1}; MLSS and 10W>MLSS respectively). R_a increased in the first 5 minutes of exercise at MLSS (6.78 ± 0.46 mg·kg^{-1}·min^{-1}) and peaked at 40min of exercise (7.62 ± 0.86 mg·kg^{-1}·min^{-1}). R_a was maintained with exercise duration until exercise termination at which point R_a was 6.42 ± 1.22 mg·kg^{-1}·min^{-1}. In contrast, R_a increased during the first 5 minutes exercise above the MLSS when it reached peak concentrations (11.01 ± 1.05 mg·kg^{-1}·min^{-1}). R_a remained significantly higher than exercise at the MLSS until exercise termination at which point R_a was 8.98 ± 2.01 mg·kg^{-1}·min^{-1}.

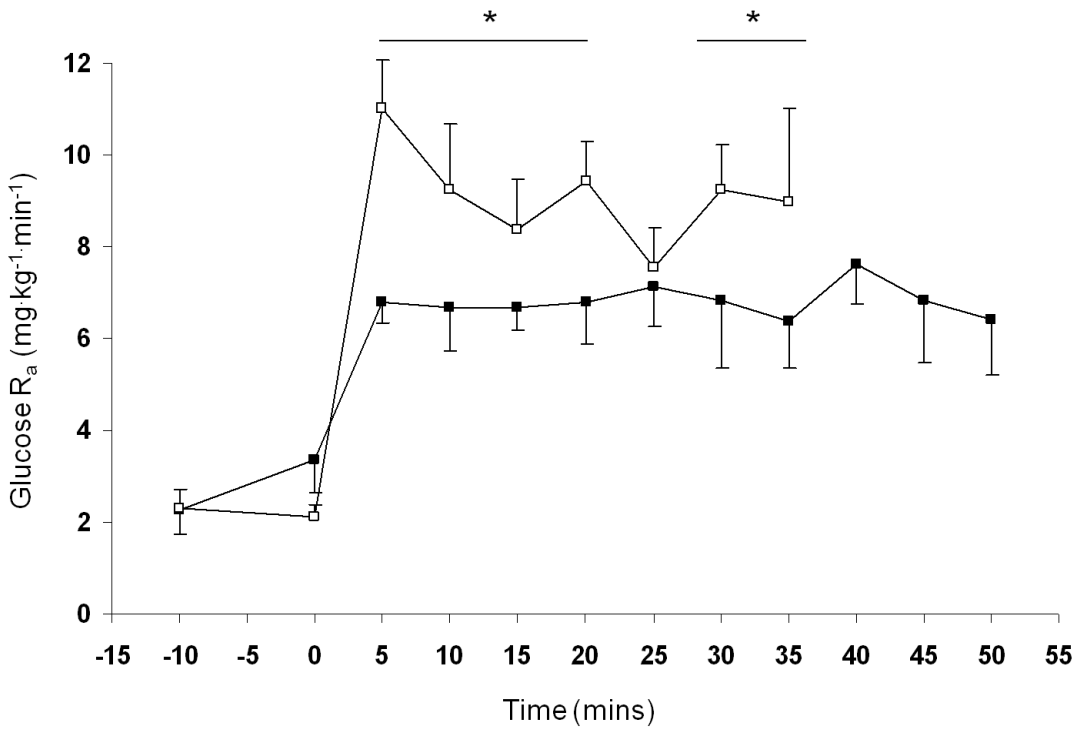

Figure 7.8. Glucose rate of appearance (R_a) during exercise to exhaustion at and above the MLSS (* P < 0.05).

Basal glucose R_d was the same between trials (3.84 ± 0.74 and 2.59 ± 0.42 $mg \cdot kg^{-1} \cdot min^{-1}$; MLSS and 10W>MLSS respectively). R_d increased to a peak value in the first 5 minutes of exercise at MLSS (8.36 ± 1.30 $mg \cdot kg^{-1} \cdot min^{-1}$). R_d remained elevated until exercise termination at which point R_d was 8.42 ± 1.06 $mg \cdot kg^{-1} \cdot min^{-1}$. In contrast, R_d increased to a peak value in the first 5 minutes exercise above the MLSS (11.47 ± 1.86 $mg \cdot kg^{-1} \cdot min^{-1}$). R_d remained elevated until exercise termination at which point R_d was 9.64 ± 1.94 $mg \cdot kg^{-1} \cdot min^{-1}$.

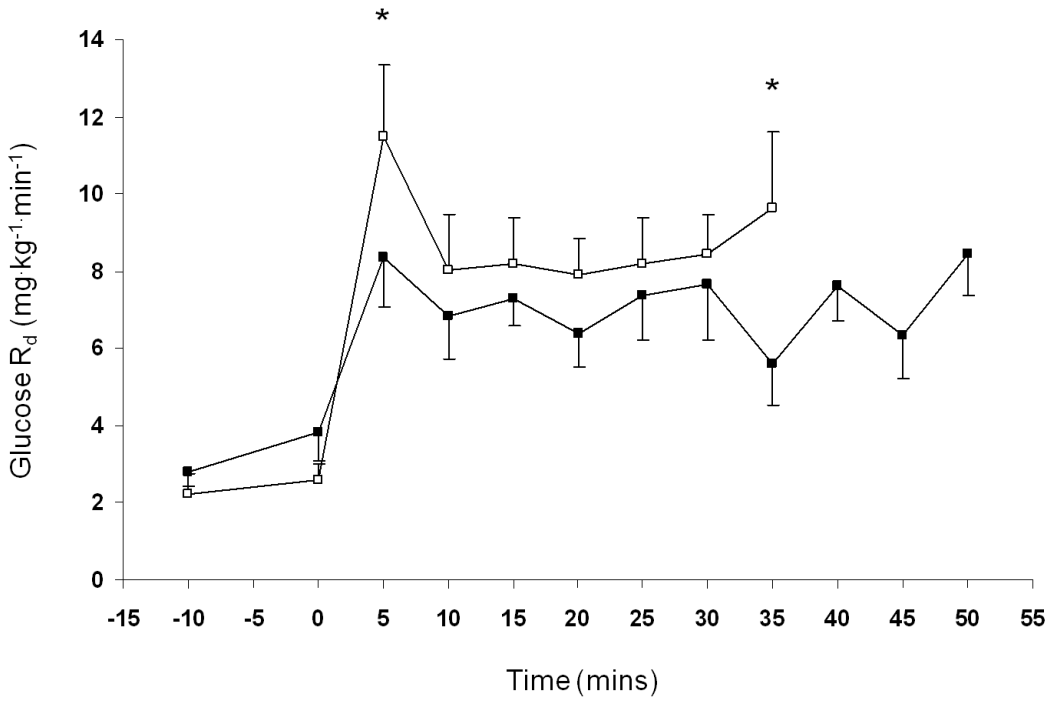

Figure 7.9. Glucose rate of disappearance (R_d) during exercise to exhaustion at and above the MLSS (* P < 0.05).

Basal glucose MCR was the same between trials (5.39 ± 0.79 and 4.29 ± 1.51 mg·kg^{-1}·min^{-1}; MLSS and 10W>MLSS respectively). MCR increased in the first 5 minutes of exercise at MLSS (9.68 ± 1.14 mg·kg^{-1}·min^{-1}) and peaked at 15min of exercise (10.68 ± 1.16 mg·kg^{-1}·min^{-1}). MCR then reached a plateau until exercise termination at which point MCR was 11.56 ± 1.41 mg·kg^{-1}·min^{-1}. In contrast, MCR increased to a peak value in the first 5 minutes of exercise at MLSS (16.06 ± 2.00 mg·kg^{-1}·min^{-1}). MCR remained elevated compared to exercise at the MLSS until exhaustion, at which point MCR was 13.56 ± 2.08 mg·kg^{-1}·min^{-1}.

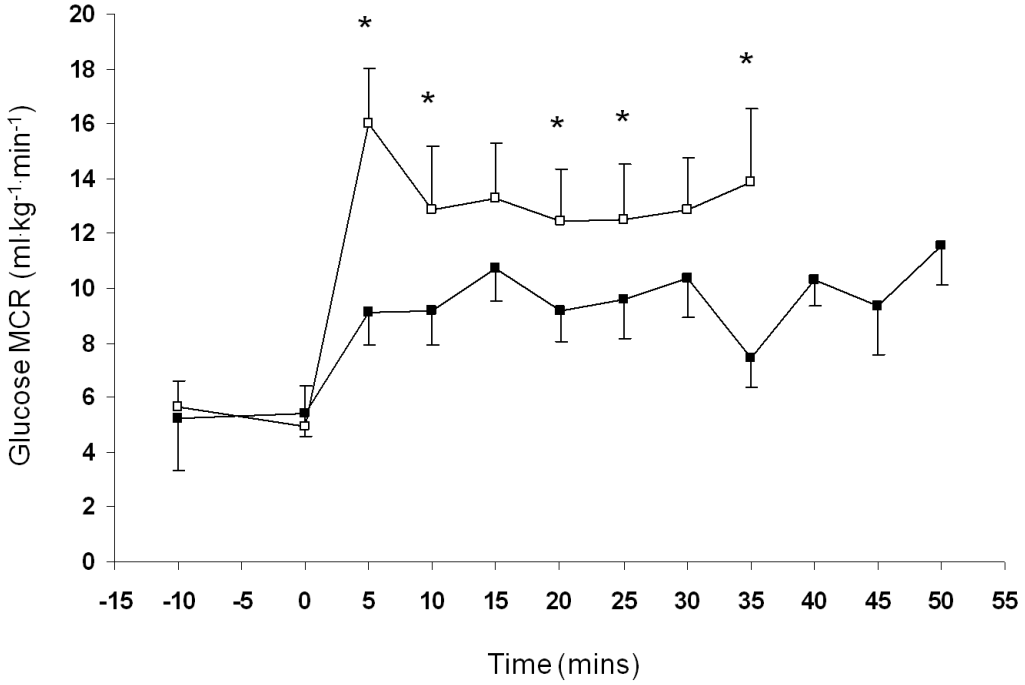

Figure 7.10. Glucose metabolic clearance rate (MCR) during exercise to exhaustion at and above the MLSS (* P < 0.05).

7.4. Discussion

This is the first study to examine lactate and glucose kinetics during exercise at and above the MLSS. The data suggests that the increase in systemic lactate concentrations during exercise 10W>MLSS was due to a disproportionate increase in lactate appearance, compared to lactate disappearance and MCR. Glucose R_a was also significantly elevated during the increased exercise intensity. However glucose R_a and R_d did not follow the same trend as lactate turnover, suggesting that the principal mechanism for increased lactate formation was from intracellular glucose.

Our data adds support to the previous suggestion by Billat et al., (2003) that the MLSS appears to represent the upper limit at which oxidative phosphorylation can account for the energetic cost of exercise. This hypothesis is supported by

the evidence presented in chapters III and IV of this thesis showing that (1) the increase and maintenance of Lactate and glucose R_a is proportional or exceeded by R_d during exercise at the MLSS. (2) there is a net balance in the lactate to pyruvate ratio at the MLSS, suggestive that adequate glycolytic supply to the TCA cycle is occurring during exercise, indicating that the pyruvate dehydrogenase (PDH) complex is not saturated (Spriet et al., 2000, Bowtell, 2007), and finally (3) a balance, followed by gradual decline is observed in RER during exercise at the MLSS, suggesting that CHO (glucose/lactate) and FFA partitioning can meet the energetic cost of exercise, supported by the net balance in glucose turnover at the MLSS.

The values reported here for lactate and glucose turnover are in agreement with previously published data corresponding to similar exercise intensities (Bergman et al., 1999a, Bergman et al., 1999b, Carter et al., 2001, Phillips et al., 1996, Williams et al., 1995, Henderson et al., 2004, Henderson et al., 2007). Interestingly, even at the termination of exercise, neither lactate nor glucose R_a increases significantly from the value observed after 10 mins of exercise when a steady state is achieved. This would suggest that exercise duration at the MLSS is not compromised by systemic supply and/or utilization of lactate or glucose. This observation is in accordance with two recent reports by Baron and coworkers (Baron et al., 2003, Baron et al., 2008) who demonstrated that arterial lactate concentrations were maintained between minutes 20 and exhaustion during exercise at the MLSS, indicating either reduced lactate production or increased peripheral utilization, despite the incidence of fatigue. The authors suggest that the absence of a progressive increase in the arterial lactate rules out lactate as a cause of exercise termination during exercise at

the MLSS. The data in the present study, as well as in the previous chapter would support this observation.

In contrast to the previously reported work of Baron and colleagues (Baron et al., 2003, Baron et al., 2008) who attempted to examine the mechanisms of fatigue at the MLSS. The present study aimed to examine the mechanisms of fatigue when exercise intensity exceeded the MLSS and as such moved into the severe exercise intensity zone (Jones and Carter, 2000). An intensity 10W > MLSS was used as previous chapters in this thesis have shown that this elicited clear elevations in circulating lactate concentrations and significantly reduced exercise time to exhaustion when compared to exercising at the MLSS. Our data suggests that the increase in systemic lactate concentrations during exercise 10W > MLSS was due to a disproportionate increase in lactate appearance, compared to lactate disappearance and MCR. Glucose R_a was also significantly elevated during the higher exercise intensity, suggesting that the principal mechanism for increased lactate formation was either (I) via increased glycolytic rate, or (II) elevated levels of glycogenolysis.

The principal mechanism for the disruption in net lactate balance during exercise above the MLSS appears to the attainment of maximal lactate clearance/oxidation processes. Lactate exchange across working muscle beds is known to be influenced by a number of factors including catecholamine release, glycogen content, blood flow, glucose, lactate and lipid transporter protein content, muscle phenotype alterations and systemic and muscle metabolites concentrations (Brooks, 1993). Unfortunately, to date, there is limited information regarding each of these factors and exercise at and above

the MLSS (Billat et al., 2003). Catecholamine concentrations have previously been reported to increase with exercise duration during 30min exercise at the MLSS (Baron et al., 2003) correlating with arterial pH and ammonia concentrations, but not lactate which remained constant during minutes 10-30 of exercise. Baron et al (2003) did not examine exercise responses above the MLSS and so there is no information available regarding how this may have differed between the exercise trials in the present study.

Catecholamine release has long been known to be tightly related to both the intensity (Galbo et al., 1975, Galbo et al., 1976) and duration of exercise (Kindermann et al., 1982, Schnabel et al., 1982). Further it has been demonstrated that both epinephrine and norepinephrine concentrations correlate with an increase in the relative power of exercise (Kindermann et al., 1982, Schnabel et al., 1982). Recently, Manetta and colleagues (Manetta et al., 2005) demonstrated that 50min cycling at a power output 15% greater than the ventilatory threshold (VT) significantly increased norepinephrine levels when compared to exercise at the VT (Manetta et al., 2005). In a similar study, Poole and colleagues (Poole et al., 1988) demonstrated that exercise above the critical power resulted in significant increases in catecholamine levels compared to exercise at critical power. Given the close correlation between power output and catecholamine release (Kindermann et al., 1982), and the sensitivity of the sympathoadrenal system to exercise transition zones (Manetta et al., 2005), it could be hypothesized that there would be a significant elevation in catecholamine responses to exercise above the MLSS, and that this may mediate the elevated glucose turnover at this exercise intensity (Watt and Hargreaves, 2002).

The influence of muscle glycogen concentration on endurance exercise capacity has been known since the early studies of Bergstrom and coworkers in the 1960's (Bergstrom et al., 1967). In a series of studies, it was elegantly demonstrated that exercise of increasing duration and intensity results in a progressive utilization of muscle glycogen stores in a fibre-type dependant manner (Gollnick et al., 1973a, Gollnick et al., 1973b, Gollnick et al., 1974, Gollnick et al., 1972). Further, exercise at higher relative intensities resulted in the progressive recruitment of type IIA and IIB fibres, which appeared to influence substrate utilization (Pernow and Saltin, 1971), metabolite accumulation (Brooks, 1991) and has been directly implicated in fatigue (Chin and Allen, 1997). Catecholamine release has been directly implicated in the enhanced glycogenolysis during prolonged exercise (Watt and Hargreaves, 2002) with the depletion thought to directly modulate the transcriptional activity of metabolic genes co-ordinating substrate utilization and mitochondrial metabolism (Hargreaves, 2004). There is no data regarding glycogen depletion at the MLSS. Brickley and colleagues (Brickley et al., 2007) demonstrated that glycogen content was reduced to approximately half resting content following 30min exercise at a work intensity corresponding to the critical power. Whilst not directly proportional, there has been suggestion that the critical power and MLSS represent a similar zone of exercise intensity (Pringle and Jones, 2002). This would support the hypothesis that prolonged exercise at and above the MLSS would result in dramatic changes in muscle glycogen and the metabolic pathways it regulates.

As previously described, the transport of lactate from cellular sites of production towards sites of removal is facilitated primarily by the MCT transport proteins

MCT1 and MCT4 (Juel and Halestrap, 1999). The tracer data presented in this chapter suggests that the increase in systemic lactate during exercise above the MLSS is due to a disproportionate increase in lactate R_a compared to R_d, coupled with an elevated glucose turnover. MCT1 has a high affinity for lactate (kM = 3.5mM) and as such regulates the uptake of lactate into the cytosolic compartment (Juel and Halestrap, 1999). Potentially, the increase in systemic lactate could therefore also be mediated by suppression of the MCT1 protein during exercise. A number of mechanisms have been shown to regulate lactate transport kinetics in *in vitro* and *ex vivo* models. Watt and colleagues (Watt et al., 1988) were amongst the first to demonstrate in a perfused rat hindlimb model, that lactate transport displayed a negative relationship with pH, and that lactate uptake was negated when pyruvate concentrations were elevated (Watt et al., 1988). Juel and colleagues used the sarcolemmal vesicle isolation preparation to show that intracellular reductions in pH could inhibit lactate influx by 50% (Juel et al., 1994), further confounding the association of lactate transporter function and pH gradients. Further work from the Juel group (Pilegaard et al., 1995) and Gladden and coworkers (Gladden et al., 1994, Watt et al., 1994, Gladden, 1991) showed the importance of blood flow for lactate transport in rodent and canine perfusion models respectively.

Most recently, Becker et al (2004) demonstrated that MCT1 worked in partnership with the sodium bicarbonate transporter (NBC) to regulate lactate uptake in oocyte preparations (Becker et al., 2004). This data would suggest that $NaHCO_3$ levels regulate MCT1 function and further support the role for pH in lactate turnover kinetics. As demonstrated by Baron et al (Baron et al., 2003, Baron et al., 2008), and reported in the previous chapter of this thesis, exercise

at and above the MLSS results in transient changes in lactate, glucose, pyruvate, pH and $NaHCO_3$. Given the importance of these factors in MCT1 regulation *in vitro* and *ex vivo*, it could be that MCT1 function was suppressed via substrate saturation or cellular concentration gradient modulation (Roth and Brooks, 1990), and that this may have compromised lactate turnover at work intensities above the MLSS. Unfortunately there is no data regarding blood flow at the MLSS, however MacPhee and colleagues (MacPhee et al., 2005) recently demonstrated that the transition from low intensity exercise to 90% of the lactate threshold elicited significant increases in leg blood flow coupled with disproportionate increases in muscle O_2 saturation. It could therefore be hypothesized that the transition from exercise at the MLSS to an intensity 10W>MLSS could have altered regional blood flow and O_2 saturation, which in turn may have influenced lactate turnover and metabolism during the exercise trials.

The increases in glucose turnover during exercise above the MLSS are in agreement with the respiratory exchange ratio data reported, which demonstrated that CHO utilization was increased during exercise above the MLSS. This would suggest that the increase in exercise intensity above the MLSS resulted in an increase in glycolytic rate or enhanced glycogenolysis. The increased glucose turnover is consistent with previous research (Bergman et al., 1999a, Van Hall et al., 2003, Williams et al., 1995) demonstrating an increase in glucose Ra with progressive increases in exercise intensity. In a similar manner to the lactate kinetic response, it appears that the increase in glucose R_a between the MLSS and 10W>MLSS was greater than the increase

in glucose R_d, thus leading to a net increase in systemic glucose and potentially the increased lactate R_a 10W>MLSS.

Whilst the tracer information presented appears to offer an explanation for the increase in lactate turnover above the MLSS, our data does little to offer a clear explanation for fatigue at the MLSS. Baron et al., (2008) suggest that the lack of a clear physiological marker to determine fatigue at the MLSS supports the notion of a regulatory, central governor during endurance exercise, as proposed by Noakes and colleagues in a number of studies (Noakes, 2007). Unfortunately, however, Baron et al., (2008) provide no evidence to support this hypothesis as there is no data to assess central drive or muscle recruitment in their study. Further, the central governor hypothesis has come under criticism (Shephard, 2009b, Shephard, 2009a) due to a lack of scientific support and a large amount of anecdotal evidence being used to generate the concept. What is often overlooked is Noakes's postulation that a peripheral governor is required to signal the localized stress of exercise in a feedback role to the central brain regulator. We (Philp et al., 2005) and others (Brooks, 2002, Hashimoto et al., 2007) have previously suggested that lactate may function in such a role, whilst reactive oxygen species (Scheele et al., 2009), myokines (Scheele et al., 2009), inorganic phosphate (Westerblad et al., 2002) and glycogen (Rauch et al., 2005) have also been suggested to fit such a mechanism. Whilst the present study offers some insight into the regulation of exercise sustainability at the MLSS, without a detailed investigation of muscle derived factors during the exercise bout, attempting to explain whole body responses is difficult. Future research should aim to examine limb substrate

kinetics and metabolite content in an attempt to link systemic and local factors during exercise to fatigue at and above the MLSS.

In conclusion, the present study demonstrates that the increase in lactate R_a during exercise at the MLSS compared to 10W>MLSS superseded the increase in lactate R_d and MCR, thus leading to a net increase in systemic lactate during exercise >10W MLSS. Further, glucose R_a demonstrated a similar response, suggesting that the elevated glucose R_a could account for the increased lactate R_a. Further research should investigate leg substrate kinetics during exercise at and above the MLSS to compliment the systemic data presented here, whilst measurements of muscle substrate stores, metabolite content and blood flow would provide valuable information about the mechanisms of fatigue during exercise at and above the MLSS.

CHAPTER VIII – GENERAL DISCUSSION

8.1. Principal Findings

Research in the past twenty years has identified the MLSS as the highest exercise intensity at which systemic lactate concentrations remain in balance during prolonged submaximal exercise (Billat et al., 2003). Functionally, homeostasis of lactate, by regulating the processes involved in turnover (i.e. rates of appearance and disappearance) appears pivotal to the maintenance of exercise performance, as exceeding the MLSS results in rapid fatigue (Billat et al., 2003). MLSS also has a practical importance as an individual's running speed or power output at the MLSS is a strong predictor of endurance performance (Jones and Carter, 2000, Jones and Doust, 1998). Therefore, the MLSS is currently viewed as the optimal measure of endurance capacity for athletes competing in events exceeding 3000m (Billat et al., 2003).

Given the potential importance of the MLSS and its applications, it is surprising the lack of knowledge regarding the changes in the physiological mechanisms which regulate this exercise intensity. It was therefore the initial aim of this thesis to examine the sensitivity of the MLSS to time of day variation and endurance training. The second aim was to examine substrate turnover and certain ions implicated in exercise function during exercise to exhaustion at and above the MLSS and investigate aspects of metabolic stress which might be associated with fatigue.

Chapter four demonstrated that the lactate concentration at the MLSS (cMLSS) was dependant on time of day, mirroring the circadian variation in body temperature. Mean lactate concentrations were significantly higher during exercise at 18:00, compared to 06:00 even though the power output remained the same in both trials. The results presented in chapter four demonstrate that individuals are capable of maintaining the power output at the MLSS (wMLSS) despite significant variation in the systemic concentration at MLSS (cMLSS). Accordingly, this observation dissociates the absolute lactate concentration from the physiological responses at MLSS in accordance with the previous studies of Baron and colleagues who suggested that lactate might not be responsible for exercise termination at the MLSS (Baron et al., 2008). The data presented in chapter four also suggested that despite the MLSS representing the theoretical upper limit of lactate transport capacity, this process has a certain degree of flexibility, as even at the higher lactate concentrations, the establishment and maintenance of a steady state was not compromised.

A further observation of chapter four was the clear dissociation between the cMLSS and a 4 $mmol \cdot l^{-1}$ lactate concentration value (OBLA) with diurnal variation. A 4 $mmol \cdot l^{-1}$ blood reference value was originally hypothesized as a valid estimate of the MLSS and termed OBLA by Heck and colleagues (Heck et al., 1985). OBLA has however received criticism in its validity in estimating the MLSS due to the intra and individual variability in cMLSS (Jones and Carter, 2000). In contrast, recent research has suggested that OBLA does offer a valid estimate of MLSS (Figueira et al., 2008, Denadai et al., 2004, Denadai et al., 2005). The data presented in

chapter four would also question the use of a blood reference value for estimating the MLSS due to the variability in cMLSS. Further, these results would also suggest that care should be taken in using a reference lactate value to monitor training session based on the MLSS, again due to both the subject variation and the cMLSS variations with time of day.

Chapter five demonstrated that in addition to being used as a marker of endurance capacity, the running speed at the MLSS can also be an effective endurance training stimulus. Chapter five also compared endurance training at the MLSS, compared to exercise intensities above and below the MLSS. Whilst both approaches produced significant improvements in the running speed at LT, MLSS and improved $\dot{V}O_2$max, there was no added improvement in training above the MLSS. It should also be noted that the subjects only performed two training sessions per week and so completed a relatively low training load. When comparing the training adaptation in the present study to studies which used a greater exercise volume or greater training period (Jones and Carter, 2000), the efficiency of training at the MLSS is evident. Interestingly, a recent study by De Mello and co-workers has shown that the intensity at the MLSS is an effective training intensity for the treatment and management of glucose intolerance in a pharmacological rodent model of type I diabetes. Following injection of alloxan, rats were trained at a swimming speed corresponding to the MLSS for 12 weeks (5 sessions/week). Post training, there was an improvement in insulin sensitivity and an increase in glucose utilization following an oral glucose tolerance test (Soares

de Alencar Mota et al., 2008). This would suggest that the MLSS might be a beneficial training intensity for both athletic and clinical populations.

Identifying the mechanisms responsible for the improvement in running speed at the MLSS was unfortunately beyond the scope of this thesis. Based on the reduction in absolute lactate concentrations pre and post training, it is likely that the training period increased the protein content of the lactate transporters MCT1 and MCT4, which increased lactate clearance capacity as previously reported for rodents (Donovan and Brooks, 1983) and humans (Bergman et al., 1999). Endurance training has also been shown to increase the protein content and activity of enzymes involved in glycolysis, B-oxidation, TCA cycle and the electron respiratory chain, collectively leading to increase oxidative phosphorylation post training (Hood et al., 2006).

A number of research studies have suggested that fartlek/redlining or interval sessions are effective models in endurance training programs, due to the increased exercise intensity achieved compared to constant intensity exercise (Jones and Carter, 2000). A fartlek/redlining approach was used in chapter five as it provided less variance from the MLSS intensity compared to high intensity interval sessions, thus allowing a valid comparison to the at MLSS training intensity. As noted, the fartlek/redlining approach did not provide an additive stimulus compared to the continuous training approach. Accordingly, the conclusion from chapter 5 was that training at the MLSS is an effective training approach. However, the possibility still remains that high intensity training

sessions, based on the MLSS may also be an effective training approach, warranting further investigation.

If MCT content is indeed an important mediator of the MLSS, then training strategies to increase MCT protein expression would be the most relevant for training application. Evertsen and colleagues (Evertsen et al., 2001) were amongst the first to suggest the effectiveness of high intensity interval training for mediating MCT1 adaptation. Twenty elite junior cross-country skiers trained for five months at intensities corresponding to 65% $\dot{V}O_2$max (moderate) or 85% $\dot{V}O_2$max (high). Following the training period the moderate training group decreased their MCT1 expression, whilst the high training group maintained total MCT1 content. Importantly, only the high training group improved running speed at the lactate threshold. More conclusive support for high intensity sprint training (HIT) has been provided by Gibala and co-workers who, in a series of studies, used a sprint interval training (SIT) model in comparison to traditional endurance training to demonstrate the potent nature of SIT (Burgomaster et al., 2007, Burgomaster et al., 2006, Burgomaster et al., 2008, Burgomaster et al., 2005). Burgomaster and colleagues demonstrated that SIT over a 6-week period increased the protein content of GLUT4, MCT1, MCT4 and cytochrome-c and the activity of PDH. Collectively this adaptation led to increased cycle time trial performance and increased glucose uptake during steady state exercise following the training period (Burgomaster et al., 2007, Burgomaster et al., 2006, Burgomaster et al., 2008, Burgomaster et al., 2005). Recently, Gibala and colleagues have reported that SIT, despite the high intensity of contraction, targets kinases associated with endurance

exercise. Specifically, AMPK and p38 MAPK phosphorylation was increased following four sprint training bouts, whilst expression of the transcriptional co-activator, PGC-1α was increased 3h post exercise (Gibala et al., 2009). In contrast, the phosphorylation of protein kinase B (PKB) or the ribosomal protein S6K1 were unchanged, re-enforcing that the SIT bout and the recovery period post SIT increases the activity of proteins and transcription factors involved in the endurance training response (Gibala et al., 2009). Benton and colleagues have recently shown that electroporation of PGC-1α in rat skeletal muscle increases lactate uptake and mediates increases in MCT1 protein content (Benton et al., 2008). This would suggest that MCT1 is regulated by PGC-1α, and therefore provides a mechanistic link to the MCT1 increases observed following SIT by Burgomaster and colleagues (Burgomaster et al., 2008, Burgomaster et al., 2005).

Chapter six used exercise to exhaustion at and 10W> MLSS as a model to profile the metabolic and respiratory responses at the two exercise intensities. In accordance with the results of Baron and colleagues (Baron et al., 2003, Baron et al., 2008), the data presented in chapter six suggests that the MLSS represents the highest exercise intensity at which systemic buffering capacity is equal to the rate of ion (Lactate, hydrogen, potassium) release. Importantly, as exercise exceeded the MLSS, there was a disproportionate increase in lactate appearance, coupled with a decrease in HCO_3^- and pCO_2 concentrations. The net result of these changes was a reduction in systemic pH and base excess, suggesting a significant increase in metabolic acidosis during exercise 10W>MLSS which could not be compensated for indefinitely.

A second important finding in chapter six was the observation that the increase in the lactate to pyruvate ratio when exercising at 10W>MLSS was mediated solely via an increase in systemic lactate, as pyruvate concentrations were unchanged between the two exercise trials. This data provides indirect evidence that lactate formation 10W>MLSS is due to glycolytic rate exceeding pyruvate oxidation (Spriet et al., 2000). This would therefore suggest that lactate is formed via the conversion of excess pyruvate, which is unable to undergo carboxylation in the PDH complex (Spriet et al., 2000), whilst lactate supply exceeds the capacity of the intracellular lactate shuttle.

Finally, chapter seven, using the same experimental model as detailed in chapter six with the addition of stable isotope tracers, demonstrated that the mechanism for lactate increase 10W>MLSS was due to lactate R_a exceeding R_d. Further, there were no notable differences in MCR or lactate oxidation 10W>MLSS, supporting the concept that the MLSS is the highest exercise intensity at which a balance exists between lactate R_a and R_d, whilst exercise above the MLSS compromises this balance due to lactate R_a exceeding R_d. The mechanistic basis for the increase in lactate R_a appeared to be due to an elevated glucose R_a during exercise 10W>MLSS. In a similar manner to lactate turnover, the net increase in glucose R_a compared to R_d was greater 10W>MLSS than at the MLSS, resulting in an increase in glucose R_a.

Whilst chapter seven provided novel information regarding the mechanisms of lactate appearance during exercise 10W>MLSS, identifying the mechanisms of

fatigue at the MLSS appears to be more complex that substrate turnover alone. In agreement with the plasma lactate concentration data provided by Baron and colleagues (2008), lactate R_a decreased during exercise to exhaustion at the MLSS. This supports the suggestion that systemic lactate concentrations to not correlate with fatigue or perceived exertion during prolonged exercise (Green et al., 2005). A limitation of this research was the fact that only systemic alterations in lactate turnover were measured and so there is no data regarding muscle substrate or ion concentrations. Given the suggestion that exercise induced fatigue is a combination of muscle and central mechanisms (McKenna and Hargreaves, 2008), future investigation should focus on both of these factors to fully elucidate fatigue at and above the MLSS.

8.2. Mechanisms regulating exercise at and above the MLSS

As detailed in chapter two, the precise mechanisms for fatigue during prolonged, submaximal exercise are currently unknown. The most logical explanation is that fatigue involves multiple systems (McKenna and Hargreaves, 2008), that one system is not the sole reason for fatigue (Miller, 2008) and specifically with regard to ion muscle regulation, that multiple flux in SID contribute to muscle fatigue (Cairns and Lindinger, 2008). With regard to the MLSS, Figure 8.1 details potential mechanisms contributing to fatigue at and above the MLSS.

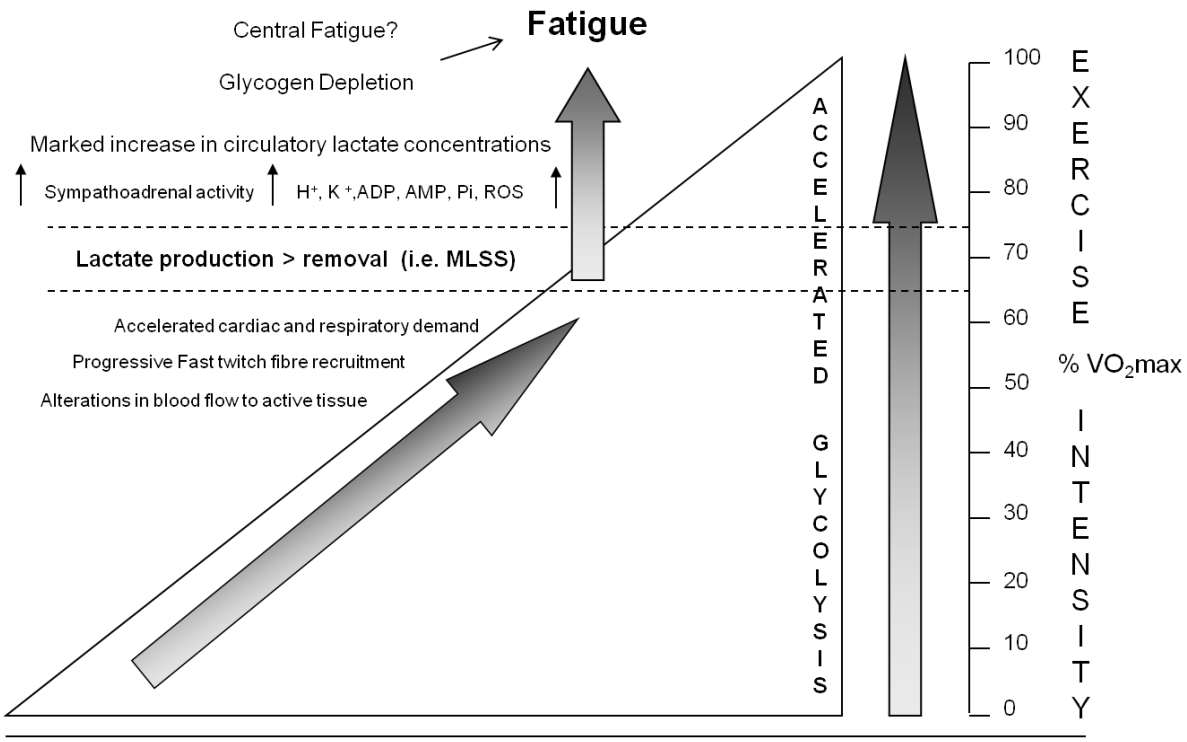

Figure 8.1. Peripheral and local factors which regulate the metabolic responses during exercise at and above the MLSS

8.2.1. Reactive oxygen species (ROS) accumulation during exercise

Muscle contraction alters the physiological milieu in skeletal muscle which results in higher levels of ROS production and oxidative stress (Ferreira and Reid, 2008). Physiological triggers of ROS production are thought to be alterations in temperature, altered O_2 and CO_2 tension and decreased pH (Powers and Jackson, 2008), however the direct mechanisms by which ROS are produced in skeletal muscle in response to exercise are currently poorly understood (Ferreira and Reid, 2008).

Importantly, the sites of generation for ROS in skeletal muscle are closely located to important functional sites for muscle metabolism and contraction. The sarcolemma and T tubules contain the enzyme NAD(P)H oxidase which produces ROS in close proximity to ion channels of the t-tubular and the SR terminal cisternae and promotes SR calcium release (Ferreira and Reid, 2008). At high ROS concentrations, such as following intense muscle contraction, there is clear evidence that ROS can directly affect biochemical processes involved with muscle contraction. Such ROS mediated modification includes lipid peroxidation, oxidation of mitochondrial and nuclear DNA, heme oxidation, tyrosine nitration, protein carbonylation and thiol oxidation (Ferreira and Reid, 2008). Of these modifications, the generation of the thiol moiety (-SH) can undergo reversible, covalent reactions with muscle derived oxidants to form disulfide bonds. Thiol oxidation can alter protein function by interfering with biochemical reactions by effecting protein structure and the availability of regulatory sites. A number of important contractile proteins have been shown to undergo reversible thiol-disulfide interactions such as the ryanodine receptor Ca^{2+} release channel (Liu and Pessah, 1994), SR Ca^{2+} ATPase (Xu et al., 1997), troponin (Putkey et al., 1993), tropomyosin (Williams and Swenson, 1982), myosin (Ajtai et al., 1989), actin (Canton et al., 2004) and the Na^{+}/K^{+} -ATPase pump (Ferreira et al., 2008).

With regard to submaximal exercise performance, there is a growing number of reports to indicate that administration of the antioxidant *N*-acetylcysteine (NAC) is able to delay muscle fatigue during submaximal exercise in humans. McKenna and colleagues have previously shown that endurance time to fatigue can be improved

by 24% during cycle exercise to exhaustion (McKenna et al., 2006). This observation supports studies in isolated muscle which has suggested ROS accumulation is central in contraction induced fatigue (Powers and Jackson, 2008). It would be of interest to measure ROS accumulation during exercise to exhaustion at and above the MLSS, whilst NAC administration at both intensities would offer a valuable investigation as to the role of ROS accumulation in MLSS fatigue.

8.2.2. Inorganic phosphate (Pi) accumulation and Phosphocreatine (PCr) recovery rate during submaximal exercise

Fryer et al., (1995) were the first to postulate that a decline in the amount of stored calcium in the SR may be instrumental in the decline in myoplasmic Ca^{2+} and subsequent fatigue with muscle contraction (Fryer et al., 1995). The key to this mechanism was the observation that accumulation of inorganic phosphate (P_i) in the SR resulted in precipitation of Ca^{2+} and P_i from the SR which ultimately resulted in a reduced amount of Ca^{2+} to be released (Allen et al., 2008). Subsequent research by Westerblad et al., (1996) utilizing phosphate injection in isolated skinned rat muscle fibres demonstrated that P_i resulted in a significant decline in SR Ca^{2+} release (Westerblad and Allen, 1996). However, the most conclusive evidence to support the P_i – SR Ca^{2+} hypothesis is the work by Dahlstedt *et al*., (2000) who utilised a transgenic mouse model deficient in creatine kinase ($CK^{-/-}$). Importantly, these mice show a diminished muscle P_i during fatigue and importantly a slower rate of myoplasmic Ca^{2+} decline during fatigue (Dahlstedt et al., 2000).

Jones and colleagues have recently demonstrated that exercise above individual critical power results in significant phosphocreatine depletion during single leg extensor exercise to exhaustion. Importantly, exercise at a power output 10% below CP resulted in a steady state in the PCr response. Added to the decline in PCr >CP was a progressive increase in the muscle content of inorganic phosphate (Pi) and a progressive decrease in pH (Jones et al., 2008). Richardson and colleagues have demonstrated that the increase in Pi during progressive exercise is not due to oxygen availability (Haseler et al., 2007, Haseler et al., 2004). Further, these studies suggest that oxidative phosphorylation is the rate limiting step in exercise at this intensity, and the decrease in PCr, increase in Pi and the reduction in pH are as a result of an imbalance in ATP re-synthesis due to the elevated demand of exercise (Jubrias et al., 2003).

8.2.3. Glycogen depletion

The influence of muscle glycogen on endurance exercise capacity has been known since the early studies of Bergstrom and coworkers in the 1960's (Bergstrom et al., 1967). Gollnick and coworkers (1974) were amongst the first researchers to investigate the effect of exercise intensity on glycogen depletion. Importantly, this study demonstrated two unique findings. Firstly, that glycogen depletion increased in proportion to exercise intensity, with a 7-fold increase in depletion observed at 84% $\dot{V}O_2$max compared to 30% $\dot{V}O_2$max. And secondly, that there was a fibre type pattern in the rate of glycogen depletion with exercise. This recruitment pattern meant that the oxidative type 1 fibres were the first to utilize glycogen stores at all exercise intensities tested, with type IIA and IIB fibres progressively

recruited as exercise duration and/or intensity increased. Interestingly, the authors also observed a strong, inverse relationship between glycogen content and lactate appearance, so that lactate was at its highest during the high intensity trial when glycogen depletion was greatest (Gollnick et al., 1974).

Vollestad et al., (1984) later added to the initial observations of Gollnick and colleagues by demonstrating that during constant load exercise at 75% $\dot{V}O_2$max there was a dual recruitment of type I and IIA fibres at the onset of exercise, whilst recruitment of type IIAB and IIB were unchanged at the onset of exercise. As exercise progressed towards exhaustion there was a gradual increase in IIAB and IIB recruitment, suggestive of physiologically different recruitment patterning between skeletal muscle subtypes (Vollestad and Blom, 1985, Vollestad et al., 1989, Vollestad et al., 1992, Vollestad et al., 1984).

Further, exercise at higher relative intensities resulted in the progressive recruitment of type IIA and IIB fibres, which appeared to influence substrate utilization (Pernow and Saltin, 1971), metabolite accumulation (Brooks, 1991) and has been directly implicated in fatigue (Chin and Allen, 1997). Catecholamine release has been directly implicated in the enhanced glycogenolysis during prolonged exercise (Watt and Hargreaves, 2002) with the depletion thought to directly modulate the transcriptional activity of metabolic genes co-ordinating substrate utilization and mitochondrial metabolism (Hargreaves, 2004).

Pringle et al., (2003) added functional relevance to these findings when they demonstrated that the relative percentage of oxidative and glycolytic fibres showed strong correlation to the speed and amplitude of the $\dot{V}O_2$ response at the onset of constant load exercise and importantly that the percentage of IIA and IIB fibres related directly to the $\dot{V}O_2$ slow component observed at heavy and severe exercise intensities (i.e. the power output corresponding to the MLSS) (Pringle et al., 2003). There is no data regarding glycogen depletion at the MLSS. Brickley and colleagues (Brickley et al., 2007) demonstrated that glycogen content was reduced to approximately half resting content following 30min exercise at a work intensity corresponding to the critical power. Whilst not directly proportional, there has been suggestion that the critical power and MLSS represent a similar exercise intensity zone (Pringle and Jones, 2002). This would allow the hypothesis that prolonged exercise at and above the MLSS would result in dramatic changes in muscle glycogen and the metabolic pathways it regulates.

8.2.4. Alteration in central and peripheral blood flow

Work from the Juel group (Pilegaard et al., 1995) and Gladden and coworkers (Gladden et al., 1994, Watt et al., 1994, Gladden, 1991) have shown the importance of blood flow for lactate transport in rodent and canine perfusion models respectively. Unfortunately there is no data regarding blood flow at the MLSS, however MacPhee and colleagues (MacPhee et al., 2005) recently demonstrated that the transition from low intensity exercise to 90% of the lactate threshold elicited significant increases in leg blood flow coupled with

disproportionate increases in muscle O_2 saturation. Helge and colleagues recently demonstrated that quadriceps blood flow increased linearly with exercise intensity (Helge et al., 2007). The authors observed a progressive increase in blood flow at the three intensities measured (25, 65 and 85% W_{max}) without any indication of alterations in blood flow between the 65% and 85% exercise zones. It is difficult to extrapolate the data reported by Helge et al., (2007) to cycling exercise as the experiment was conducted using an isolated leg kicking model, however this data does question whether reduced local blood flow is a factor during prolonged exercise.

Utilizing a ^{1}H magnetic resonance spectroscopy based approach to measure myoglobin oxygenation, Richardson and colleagues suggest that at exercise intensities exceeding 60% W_{max} there is substantial oxygen reserve and delivery to active muscle (Richardson, 2000a, Richardson et al., 2006, Richardson et al., 2001). Importantly, the same research group has also demonstrated that the increase in skeletal muscle lactate efflux during progressive exercise is dissociated from cellular oxygenation (Richardson et al., 1998). Certainly, there is a growing body of research in this area to suggest that blood flow and muscle oxygenation is sufficient during submaximal exercise and there is no threshold in either parameter to accompany the metabolic stress associated with corresponding exercise intensities (Richardson, 2000b, Richardson, 2000a, Richardson et al., 2006, Richardson et al., 2001, Richardson et al., 1998).

8.2.5. The central component of exercise fatigue

Whilst the tracer information presented in chapter seven offers an explanation for the increase in lactate turnover above the MLSS, the data in this thesis does little to offer a clear explanation for fatigue at the MLSS. Baron et al., (2008) suggest that the lack of a clear physiological marker to determine fatigue at the MLSS supports the notion of a regulatory, central governor during endurance exercise, as proposed by Noakes and colleagues in a number of studies in the past decade (Noakes, 2007). However, whilst the central governor hypothesis offers an attractive explanation for exercise fatigue, there is still a lack of convincing data to justify acceptance of this theory (Shephard, 2009b, Shephard, 2009a).

Whilst Noakes has coined the phrase 'central governor', the importance of the central component of fatigue has long been recognized as important in endurance performance (Gandevia, 2001, Gandevia et al., 2008). Unfortunately, the biggest limitation to this area of research in humans is the difficulty in quantifying central fatigue during exercise in humans (Gandevia, 2001). Central fatigue can be broadly defined as a progressive exercise-induced failure to voluntarily activate skeletal muscle (Taylor and Gandevia, 2008). Evidence of this phenomenon can be demonstrated via electrical stimulation *in vivo* pre and post exhaustive exercise. Herbert and Gandevia (2002) demonstrated that superimposing a muscle contraction in the quadriceps muscle post exercise increased the force production during an MVC (Herbert et al., 2002). This was suggestive of an inability of motor unit firing signifying central fatigue, and indicating that central processes proximal to the site of motor axon stimulation are contributing to a loss of force.

Repetitive submaximal activation has been suggested to decrease the responsiveness of synaptic input which ultimately leads to weak voluntary contractions (Nordstrom et al., 2007). In combination with the reduction in synaptic input responsiveness is a change to the input received by the motorneuron (Taylor and Gandevia, 2008). The inputs that are most likely to change include reflex inputs from the motor afferents, recurrent inhibition and descending drive (Taylor and Gandevia, 2008). Firing of Golgi tendon organs is also likely to decrease with the fall in muscle force during fatiguing maximal contractions (Zytnicki et al., 1990). In particular, small diameter (groups III and IV) muscle afferents are variously sensitive to mechanical and chemical stimuli so that some increase firing with the accumulation of metabolites in the fatigued muscle (Zytnicki et al., 1990). Unfortunately, there is limited information regarding central fatigue during submaximal, whole body exercise, with the majority of studies using maximal voluntary contractions in large muscle groups such as the quadriceps, or in small muscle groups such as the wrist flexors (Gandevia, 2001). What submaximal studies have shown is that individual perceived effort of exertion is important for maintaining contractile force, and so appears to indicate the onset of central fatigue. During submaximal MVC's (15% of maximal) subjects reported a mild initial effort, which increased to a very large effort after ~40 minutes of exercise (Sogaard et al., 2006). Of interest was the fact that during this increase in perceived pain, EMG in the active muscles only increased to 35% of maximal levels, indicating clear discordance between the perception of muscle fatigue and actual mechanical fatigue. Smith et al., (2007) also identify a similar phenomenon

to occur during a prolonged 5% MVC over a 70 minute period (Smith and Newham, 2007).

The use of surface EMG electrodes has received some support for assessing muscle firing rate during submaximal cycling exercise (Rainoldi et al., 2008, Hunter et al., 2002, Oberg et al., 1994, Hary et al., 1982), however this approach has also received criticism for the accuracy of measurements, and the substantial 'noise' associated with large muscle mass exercise (Pringle and Jones, 2002). It has been suggested that a muscle recruitment threshold (EMG_{FT}) exists at power outputs exceeding the CP and that this represents alterations in fibre recruitment in the severe exercise domain. However, Pringle and Jones (2002) questioned the occurrence of the EMG_{FT}, the validity of EMG measurements during submaximal cycling exercise and the efficacy of using EMG measures to assess the CP or MLSS intensities. Certainly the data in the present thesis and the study by Baron et al (2008) suggest a reduction in central drive may be involved in the onset of fatigue at the MLSS. However, as to whether fatigue at and above the MLSS is due to the theoretical central governor remains to be seen.

8.3. Conclusion

From a practical aspect this thesis demonstrates the MLSS as a potent training intensity, which can increase submaximal (MLSS, LT) and maximal ($\dot{V}O_2max$) aspects of endurance capacity despite a marginal training load (60 min/week). Evidence is presented suggesting that the MLSS represents the highest exercise intensity at which systemic buffering capacity is equal to the rate of ion (Lactate,

hydrogen, potassium) release. As exercise exceeded the MLSS, there was a disproportionate increase in lactate appearance, coupled with a decrease in HCO_3^- and pCO_2 concentrations. Elevated lactate concentrations above the MLSS appear to be due to the rate of lactate appearance exceeding clearance capacity, supporting the fundamental basis for the MLSS as the upper limit of cell and systemic lactate balance. The net result of these changes was an increase in metabolic acidosis during exercise 10W>MLSS and a decrease in exercise capacity at this intensity.

Collectively this thesis identifies the MLSS as an important transition for submaximal endurance exercise and demonstrates that a number of physiological mechanisms involved in fatigue are increased when exercise progresses beyond this exercise intensity. The MLSS therefore has theoretical and practical function in exercise physiology as a model for exploring the mechanistic basis of muscular fatigue and the determinants of human exercise tolerance.

References

AJTAI, K., FRENCH, A. R. & BURGHARDT, T. P. 1989. Myosin cross-bridge orientation in rigor and in the presence of nucleotide studied by electron spin resonance. *Biophys J,* 56, 535-41.

ALLEN, D. G., LAMB, G. D. & WESTERBLAD, H. 2008. Impaired calcium release during fatigue. *J Appl Physiol,* 104, 296-305.

BARON, B., DEKERLE, J., ROBIN, S., NEVIERE, R., DUPONT, L., MATRAN, R., VANVELCENAHER, J., ROBIN, H. & PELAYO, P. 2003. Maximal lactate steady state does not correspond to a complete physiological steady state. *Int J Sports Med,* 24, 582-7.

BARON, B., NOAKES, T. D., DEKERLE, J., MOULLAN, F., ROBIN, S., MATRAN, R. & PELAYO, P. 2008. Why does exercise terminate at the maximal lactate steady state intensity? *Br J Sports Med,* 42, 528-33.

BENTON, C. R., YOSHIDA, Y., LALLY, J., HAN, X. X., HATTA, H. & BONEN, A. 2008. PGC-1alpha increases skeletal muscle lactate uptake by increasing the expression of MCT1 but not MCT2 or MCT4. *Physiol Genomics,* 35, 45-54.

BERGMAN, B. C., WOLFEL, E. E., BUTTERFIELD, G. E., LOPASCHUK, G. D., CASAZZA, G. A., HORNING, M. A. & BROOKS, G. A. 1999. Active muscle and whole body lactate kinetics after endurance training in men. *J Appl Physiol,* 87, 1684-96.

BERGSTROM, J., HERMANSEN, L., HULTMAN, E. & SALTIN, B. 1967. Diet, muscle glycogen and physical performance. *Acta Physiol Scand,* 71, 140-50.

BILLAT, V. L., SIRVENT, P., PY, G., KORALSZTEIN, J. P. & MERCIER, J. 2003. The concept of maximal lactate steady state: a bridge between biochemistry, physiology and sport science. *Sports Med,* 33, 407-26.

BRICKLEY, G., GREEN, S., JENKINS, D. G., MCEINERY, M., WISHART, C., DOUST, J. D. & WILLIAMS, C. A. 2007. Muscle metabolism during constant- and alternating-intensity exercise around critical power. *Int J Sports Med,* 28, 300-5.

BROOKS, G. A. 1991. Current concepts in lactate exchange. *Med Sci Sports Exerc,* 23, 895-906.

BURGOMASTER, K. A., CERMAK, N. M., PHILLIPS, S. M., BENTON, C. R., BONEN, A. & GIBALA, M. J. 2007. Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *Am J Physiol Regul Integr Comp Physiol,* 292, R1970-6.

BURGOMASTER, K. A., HEIGENHAUSER, G. J. & GIBALA, M. J. 2006. Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. *J Appl Physiol,* 100, 2041-7.

BURGOMASTER, K. A., HOWARTH, K. R., PHILLIPS, S. M., RAKOBOWCHUK, M., MACDONALD, M. J., MCGEE, S. L. & GIBALA, M. J. 2008. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol,* 586, 151-60.

BURGOMASTER, K. A., HUGHES, S. C., HEIGENHAUSER, G. J., BRADWELL, S. N. & GIBALA, M. J. 2005. Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol,* 98, 1985-90.

CAIRNS, S. P. & LINDINGER, M. I. 2008. Do multiple ionic interactions contribute to skeletal muscle fatigue? *J Physiol,* 586, 4039-54.

CANTON, M., NEVEROVA, I., MENABO, R., VAN EYK, J. & DI LISA, F. 2004. Evidence of myofibrillar protein oxidation induced by postischemic reperfusion in isolated rat hearts. *Am J Physiol Heart Circ Physiol,* 286, H870-7.

CHIN, E. R. & ALLEN, D. G. 1997. Effects of reduced muscle glycogen concentration on force, Ca2+ release and contractile protein function in intact mouse skeletal muscle. *J Physiol,* 498 (Pt 1), 17-29.

DAHLSTEDT, A. J., KATZ, A., WIERINGA, B. & WESTERBLAD, H. 2000. Is creatine kinase responsible for fatigue? Studies of isolated skeletal muscle deficient in creatine kinase. *FASEB J,* 14, 982-90.

DENADAI, B. S., FIGUEIRA, T. R., FAVARO, O. R. & GONCALVES, M. 2004. Effect of the aerobic capacity on the validity of the anaerobic threshold for determination of the maximal lactate steady state in cycling. *Braz J Med Biol Res,* 37, 1551-6.

DENADAI, B. S., GOMIDE, E. B. & GRECO, C. C. 2005. The relationship between onset of blood lactate accumulation, critical velocity, and maximal lactate steady state in soccer players. *J Strength Cond Res,* 19, 364-8.

DONOVAN, C. M. & BROOKS, G. A. 1983. Endurance training affects lactate clearance, not lactate production. *Am J Physiol,* 244, E83-92.

EVERTSEN, F., MEDBO, J. I. & BONEN, A. 2001. Effect of training intensity on muscle lactate transporters and lactate threshold of cross-country skiers. *Acta Physiol Scand,* 173, 195-205.

FERREIRA, L. F. & REID, M. B. 2008. Muscle-derived ROS and thiol regulation in muscle fatigue. *J Appl Physiol,* 104, 853-60.

FIGUEIRA, T. R., CAPUTO, F., PELARIGO, J. G. & DENADAI, B. S. 2008. Influence of exercise mode and maximal lactate-steady-state concentration on the validity of OBLA to predict maximal lactate-steady-state in active individuals. *J Sci Med Sport,* 11, 280-6.

FRYER, M. W., OWEN, V. J., LAMB, G. D. & STEPHENSON, D. G. 1995. Effects of creatine phosphate and P(i) on Ca2+ movements and tension development in rat skinned skeletal muscle fibres. *J Physiol,* 482 (Pt 1), 123-40.

GANDEVIA, S. C. 2001. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev,* 81, 1725-89.

GANDEVIA, S. C., BUTLER, J. E. & TAYLOR, J. L. 2008. Viewpoint: Fatigue mechanisms determining exercise performance: integrative physiology is systems physiology. *J Appl Physiol,* 104, 1546.

GIBALA, M. J., MCGEE, S. L., GARNHAM, A. P., HOWLETT, K. F., SNOW, R. J. & HARGREAVES, M. 2009. Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. *J Appl Physiol,* 106, 929-34.

GLADDEN, L. B. 1991. Net lactate uptake during progressive steady-level contractions in canine skeletal muscle. *J Appl Physiol,* 71, 514-20.

GLADDEN, L. B., CRAWFORD, R. E. & WEBSTER, M. J. 1994. Effect of lactate concentration and metabolic rate on net lactate uptake by canine skeletal muscle. *Am J Physiol,* 266, R1095-101.

GOLLNICK, P. D., PIEHL, K. & SALTIN, B. 1974. Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J Physiol,* 241, 45-57.

GREEN, J. M., MCLESTER, J. R., CREWS, T. R., WICKWIRE, P. J., PRITCHETT, R. C. & REDDEN, A. 2005. RPE-lactate dissociation during extended cycling. *Eur J Appl Physiol,* 94, 145-50.

HARGREAVES, M. 2004. Muscle glycogen and metabolic regulation. *Proc Nutr Soc,* 63, 217-20.

HARY, D., BELMAN, M. J., PROPST, J. & LEWIS, S. 1982. A statistical analysis of the spectral moments used in EMG tests of endurance. *J Appl Physiol,* 53, 779-83.

HASELER, L. J., LIN, A., HOFF, J. & RICHARDSON, R. S. 2007. Oxygen availability and PCr recovery rate in untrained human calf muscle: evidence of metabolic limitation in normoxia. *Am J Physiol Regul Integr Comp Physiol,* 293, R2046-51.

HASELER, L. J., LIN, A. P. & RICHARDSON, R. S. 2004. Skeletal muscle oxidative metabolism in sedentary humans: 31P-MRS assessment of O2 supply and demand limitations. *J Appl Physiol,* 97, 1077-81.

HECK, H., MADER, A., HESS, G., MUCKE, S., MULLER, R. & HOLLMANN, W. 1985. Justification of the 4-mmol/l lactate threshold. *Int J Sports Med,* 6, 117-30.

HELGE, J. W., STALLKNECHT, B., RICHTER, E. A., GALBO, H. & KIENS, B. 2007. Muscle metabolism during graded quadriceps exercise in man. *J Physiol,* 581, 1247-58.

HERBERT, R. D., MOSELEY, A. M., BUTLER, J. E. & GANDEVIA, S. C. 2002. Change in length of relaxed muscle fascicles and tendons with knee and ankle movement in humans. *J Physiol,* 539, 637-45.

HOOD, D. A., IRRCHER, I., LJUBICIC, V. & JOSEPH, A. M. 2006. Coordination of metabolic plasticity in skeletal muscle. *J Exp Biol,* 209, 2265-75.

HUNTER, A. M., ST CLAIR GIBSON, A., DERMAN, W. E., LAMBERT, M., DENNIS, S. C. & NOAKES, T. D. 2002. The effect of selective beta1-blockade on EMG signal characteristics during progressive endurance exercise. *Eur J Appl Physiol,* 88, 275-81.

JONES, A. M. & CARTER, H. 2000. The effect of endurance training on parameters of aerobic fitness. *Sports Med,* 29, 373-86.

JONES, A. M. & DOUST, J. H. 1998. The validity of the lactate minimum test for determination of the maximal lactate steady state. *Med Sci Sports Exerc,* 30, 1304-13.

JONES, A. M., WILKERSON, D. P., DIMENNA, F., FULFORD, J. & POOLE, D. C. 2008. Muscle metabolic responses to exercise above and below the "critical power" assessed using 31P-MRS. *Am J Physiol Regul Integr Comp Physiol,* 294, R585-93.

JUBRIAS, S. A., CROWTHER, G. J., SHANKLAND, E. G., GRONKA, R. K. & CONLEY, K. E. 2003. Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle in vivo. *J Physiol,* 553, 589-99.

LIU, G. & PESSAH, I. N. 1994. Molecular interaction between ryanodine receptor and glycoprotein triadin involves redox cycling of functionally important hyperreactive sulfhydryls. *J Biol Chem,* 269, 33028-34.

MACPHEE, S. L., SHOEMAKER, J. K., PATERSON, D. H. & KOWALCHUK, J. M. 2005. Kinetics of O2 uptake, leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower region of the moderate-intensity exercise domain. *J Appl Physiol,* 99, 1822-34.

MCKENNA, M. J. & HARGREAVES, M. 2008. Resolving fatigue mechanisms determining exercise performance: integrative physiology at its finest! *J Appl Physiol,* 104, 286-7.

MCKENNA, M. J., MEDVED, I., GOODMAN, C. A., BROWN, M. J., BJORKSTEN, A. R., MURPHY, K. T., PETERSEN, A. C., SOSTARIC, S. & GONG, X. 2006. N-acetylcysteine attenuates the decline in muscle Na+,K+-pump activity and delays fatigue during prolonged exercise in humans. *J Physiol,* 576, 279-88.

MILLER, B. F. 2008. No single mechanism. *J Appl Physiol,* 105, 363-4.

NOAKES, T. D. 2007. The central governor model of exercise regulation applied to the marathon. *Sports Med,* 37, 374-7.

NORDSTROM, M. A., GORMAN, R. B., LAOURIS, Y., SPIELMANN, J. M. & STUART, D. G. 2007. Does motoneuron adaptation contribute to muscle fatigue? *Muscle Nerve,* 35, 135-58.

OBERG, T., KARSZNIA, A., GARE, B. A. & LAGERSTRAND, A. 1994. Physical training of children with juvenile chronic arthritis. Effects on force, endurance and EMG response to localized muscle fatigue. *Scand J Rheumatol,* 23, 92-5.

PERNOW, B. & SALTIN, B. 1971. Availability of substrates and capacity for prolonged heavy exercise in man. *J Appl Physiol,* 31, 416-22.

PILEGAARD, H., BANGSBO, J., HENNINGSEN, P., JUEL, C. & RICHTER, E. A. 1995. Effect of blood flow on muscle lactate release studied in perfused rat hindlimb. *Am J Physiol,* 269, E1044-51.

POWERS, S. K. & JACKSON, M. J. 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev,* 88, 1243-76.

PRINGLE, J. S., DOUST, J. H., CARTER, H., TOLFREY, K., CAMPBELL, I. T., SAKKAS, G. K. & JONES, A. M. 2003. Oxygen uptake kinetics during moderate, heavy and severe intensity "submaximal"

exercise in humans: the influence of muscle fibre type and capillarisation. *Eur J Appl Physiol,* 89, 289-300.

PRINGLE, J. S. & JONES, A. M. 2002. Maximal lactate steady state, critical power and EMG during cycling. *Eur J Appl Physiol,* 88, 214-26.

PUTKEY, J. A., DOTSON, D. G. & MOUAWAD, P. 1993. Formation of inter- and intramolecular disulfide bonds can activate cardiac troponin C. *J Biol Chem,* 268, 6827-30.

RAINOLDI, A., GAZZONI, M., MERLETTI, R. & MINETTO, M. A. 2008. Mechanical and EMG responses of the vastus lateralis and changes in biochemical variables to isokinetic exercise in endurance and power athletes. *J Sports Sci,* 26, 321-31.

RICHARDSON, R. S. 2000a. Intracellular Po2 and bioenergetic measurements in skeletal muscle: the role of exercise paradigm. *Am J Physiol Regul Integr Comp Physiol,* 278, R1111-3.

RICHARDSON, R. S. 2000b. What governs skeletal muscle VO2max? New evidence. *Med Sci Sports Exerc,* 32, 100-7.

RICHARDSON, R. S., DUTEIL, S., WARY, C., WRAY, D. W., HOFF, J. & CARLIER, P. G. 2006. Human skeletal muscle intracellular oxygenation: the impact of ambient oxygen availability. *J Physiol,* 571, 415-24.

RICHARDSON, R. S., NEWCOMER, S. C. & NOYSZEWSKI, E. A. 2001. Skeletal muscle intracellular PO(2) assessed by myoglobin desaturation: response to graded exercise. *J Appl Physiol,* 91, 2679-85.

RICHARDSON, R. S., NOYSZEWSKI, E. A., LEIGH, J. S. & WAGNER, P. D. 1998. Lactate efflux from exercising human skeletal muscle: role of intracellular PO2. *J Appl Physiol,* 85, 627-34.

SHEPHARD, R. J. 2009a. Hard evidence for a central governor is still lacking! *J Appl Physiol,* 106, 343-6.

SHEPHARD, R. J. 2009b. Is it time to retire the 'central Governor'? *Sports Med,* 39, 709-21.

SMITH, I. C. & NEWHAM, D. J. 2007. Fatigue and functional performance of human biceps muscle following concentric or eccentric contractions. *J Appl Physiol,* 102, 207-13.

SOARES DE ALENCAR MOTA, C., RIBEIRO, C., DE ARAUJO, G. G., DE ARAUJO, M. B., DE BARROS MANCHADO-GOBATTO, F., VOLTARELLI, F. A., DE OLIVEIRA, C. A., LUCIANO, E. & DE MELLO, M. A. 2008. Exercise training in the aerobic/anaerobic metabolic transition prevents glucose intolerance in alloxan-treated rats. *BMC Endocr Disord,* 8, 11.

SOGAARD, K., GANDEVIA, S. C., TODD, G., PETERSEN, N. T. & TAYLOR, J. L. 2006. The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol,* 573, 511-23.

SPRIET, L. L., HOWLETT, R. A. & HEIGENHAUSER, G. J. 2000. An enzymatic approach to lactate production in human skeletal muscle during exercise. *Med Sci Sports Exerc,* 32, 756-63.

TAYLOR, J. L. & GANDEVIA, S. C. 2008. A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *J Appl Physiol,* 104, 542-50.

VOLLESTAD, N. K. & BLOM, P. C. 1985. Effect of varying exercise intensity on glycogen depletion in human muscle fibres. *Acta Physiol Scand,* 125, 395-405.

VOLLESTAD, N. K., BLOM, P. C. & GRONNEROD, O. 1989. Resynthesis of glycogen in different muscle fibre types after prolonged exhaustive exercise in man. *Acta Physiol Scand,* 137, 15-21.

VOLLESTAD, N. K., TABATA, I. & MEDBO, J. I. 1992. Glycogen breakdown in different human muscle fibre types during exhaustive exercise of short duration. *Acta Physiol Scand,* 144, 135-41.

VOLLESTAD, N. K., VAAGE, O. & HERMANSEN, L. 1984. Muscle glycogen depletion patterns in type I and subgroups of type II fibres during prolonged severe exercise in man. *Acta Physiol Scand,* 122, 433-41.

WATT, M. J. & HARGREAVES, M. 2002. Effect of epinephrine on glucose disposal during exercise in humans: role of muscle glycogen. *Am J Physiol Endocrinol Metab,* 283, E578-83.

WATT, P. W., GLADDEN, L. B., HUNDAL, H. S. & CRAWFORD, R. E. 1994. Effects of flow and contraction on lactate transport in the perfused rat hindlimb. *Am J Physiol,* 267, E7-13.

WESTERBLAD, H. & ALLEN, D. G. 1996. The effects of intracellular injections of phosphate on intracellular calcium and force in single fibres of mouse skeletal muscle. *Pflugers Arch,* 431, 964-70.

WILLIAMS, D. L., JR. & SWENSON, C. A. 1982. Disulfide bridges in tropomyosin. Effect on ATPase activity of actomyosin. *Eur J Biochem,* 127, 495-9.

XU, S., LACCOTRIPE, M., HUANG, X., RIGOTTI, A., ZANNIS, V. I. & KRIEGER, M. 1997. Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-BI, an HDL receptor that mediates selective lipid uptake. *J Lipid Res,* 38, 1289-98.

ZYTNICKI, D., LAFLEUR, J., HORCHOLLE-BOSSAVIT, G., LAMY, F. & JAMI, L. 1990. Reduction of Ib autogenetic inhibition in motoneurons during contractions of an ankle extensor muscle in the cat. *J Neurophysiol,* 64, 1380-9.