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Can Ventilation Be Used as a Non-invasive Measure of Lactate Clearance?

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Abstract

The purpose of this study was to determine whether the rate of lactate clearance can be determined non-invasively through a potential relationship with ventilation. Current research has given lactate the status of an important metabolic substrate as opposed to a fatiguing agent, however, the lactate threshold and rate of lactate clearance is still used as a physiological marker of exercise intensity and metabolic stress. Current methods used to measure lactate clearance are involved and costly therefore alternative procedures would be useful and the aim of this investigation is to establish whether a non-invasive predictor of lactate clearance has value, particularly in high intensity, team sports.

14 youth professional football players mean age 17±0.7 years, body mass 75±8 Kg and height 182±7 cm were recruited to this study. The participants completed six months of full-time professional football training before undertaking a treadmill test to volitional exhaustion at 10 Kmh^{-1} with an initial gradient of 0%, increasing by 2% every 2 minutes. Oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), ventilation ($\dot{V}_e$) and respiratory exchange ratio (RER) were recorded continuously throughout the test. The player's rating of perceived exertion (RPE) was recorded at the end of each 2 minute work rate increment using a Borg scale. Capillary blood samples were collected at rest, at peak exercise and at 1, 3, 5 and 15 minutes during an active recovery period (where players walked at 5 $km.hr^{-1}$ on a 1% gradient).The capillary blood samples were analysed immediately for blood lactate concentration (b[La]).

At the time of the maximal exercise test, $\dot{V}O_{2peak}$ was 58.8±9.9 $ml.kg^{-1}.min^{-1}$, $\dot{V}CO_{2peak}$ was 4.97±0.9 $l.min^{-1}$ and $\dot{V}_{epeak}$ was 148.65± 23.5 $l.min^{-1}$. Ventilatory threshold (VT) was derived from the $\dot{V}O_2/\dot{V}CO_2$ relationship using the V-slope method and was 62.5±8.7 % of $\dot{V}O_{2peak}$. Lactate clearance was expressed as the half-time for peak b[La] to return to resting b[La] ($T_{½}$ b[La]) and mean

$T_{½}$ b[La] was 11.7±5.7 min. There was a significant positive correlation between T½ b[La] and $\dot{V}e_{peak}$ ($r = 0.65$; $p < 0.05$). Importantly, the time course of lactate clearance was a single exponential decay in these athletes and so T½ b[La] was the best discriminator of lactate clearance.

This data demonstrates that full-time training for football may not provide an appropriate stimulus to maximise the physiological processes of lactate clearance. This is likely to have an impact on the ability of players to recover from the intermittent, high intensity, anaerobic bouts of exercise common in the sport. Peak ventilation is a significant predictor of lactate clearance in these athletes and may provide an appropriate mechanism to monitor targeted training interventions to improve lactate clearance.

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List of Abbreviations

b[La] – Blood lactate concentration.

T ½ b[La]- The half-time of blood lactate clearance. Defined as the time that it takes the b[La] to return to half of the delta value between $b[La]_{peak}$ post exercise and the observed resting b[La] measured prior to the test.

$\dot{V}$ **O_2** – Oxygen Uptake.

$\dot{V}$ **CO_2** – Expired Carbon Dioxide.

$\dot{V}_e$ – Minute Ventilation.

RER – Respiratory Exchange Ratio.

CMJ – Counter Movement Jump

Introduction – Chapter 1

The terms “lactate” and “lactic acid” are used almost interchangeably in sporting circles by coaches and athletes alike. They are generally used in association with the burning sensation felt in the working muscle as a result of high intensity exercise and, in some extremely ill-informed instances, for the Delayed Onset Muscle Soreness (DOMS) felt in the hours and days after exercise. However, the consensus amongst the scientific community is that lactate is not a worthless, fatiguing, physiological end product but a valuable substrate when used efficiently.

Prominent research has clearly illustrated the use of lactate as a substrate in highly oxidative type I muscle fibres and also within the myocardium (Gertz, 1988). This being the case, it seems that the obvious questions to ask are why it is desirable to increase the exercise intensity at which the lactate threshold occurs and what is the athletic significance of an efficient lactate removal ability? The answer lies in the fact that the accumulation of lactate is an indicator of an altered metabolic environment and its prolongation is indicative of the demand for energy and the ability of the muscle to meet it through oxidative means. Consequently, the rate at which lactate is cleared can be used as a proxy measure for the length of time it takes to return resting metabolic activity to the working muscle and therefore the capacity to undertake further, high intensity bouts of exercise. For these reasons, it is abundantly clear that, despite the fact that lactate is not a cause of fatigue, its accumulation and subsequent clearance is of great significance with regard to athletic performance. This is particularly the case in intermittent, high intensity sports which require supra-maximal bouts of exercise. Football is one such sport where a number of anaerobic efforts are interspersed through a period of activity. The recovery from these bouts and ability to participate in another may be characterised by the speed at which lactate is removed.

There is a significant body of research which has extensively investigated the relationship between exercise and ventilation from a number of different perspectives and in different populations (Casaburi & Wasserman 1991; Wasserman, 1987; Meyer, 2004; Rogbergs, 2004; Hagberg et al, 1982, Patterson et al, 1990; Maltais et al 1998, 1996). However, a common theme seems to be the difficulty in marrying the observations made in these studies with specific physiological processes. The consequence of this is a general acceptance of associations between the ventilatory responses to intense/prolonged exercise and the metabolic adjustments which occur to meet the demands of such exercise.

An example of such an instance is the "talk test" (Foster et al, 2008). This proposes that the length of time a subject can comfortably converse during an exercise test can be used to make inferences regarding the metabolic changes which occur at a cellular level. By its very nature, speech will be impaired when the ventilatory system is taxed and the extent to which it is impaired is likely to be in some way related to the subject's degree of exertion. As such, it may be moderately useful for exercise prescription in clinical and non-athletic populations (Foster et al, 2008). However, during high intensity exercise, it has been shown that there are a number of central and peripheral factors which influence ventilation (Casaburi & Wasserman, 1987; Meyer 2004) and therefore a causal relationship cannot be established between changes in ventilation and the altered physiological environment through this protocol.

The relationship between lactate clearance and ventilation presents similar difficulties. We know that both lactate clearance and ventilatory parameters contribute to athletic performance and are enhanced as a result of training. We know their efficiency relies on a number of common vehicles, circumstances and locales such as the blood, oxygen availability and working tissue respectively (Brooks, 2009; Casaburi 1991). We also know that ventilation is driven by the cellular conditions (not the converse). However, there is no established evidence base to allow discrimination between the

indirect relationship ventilation and lactate clearance share as independent transient variables during exercise and a more intimate, causal association where one directly influences the other. An effort to separate the ventilatory response and lactate balance during exercise has been made in studies focussing on patients with Mcardle's syndrome who have an inability to produce lactate yet still show a hyperventilatory response (Hagberg et al, 1982; Patterson et al, 1990). Though tempting to accept this theory, it does not account for the effect of Mcardle's syndrome on the central chemoreceptors.

More informative may be the investigation in populations suffering from COPD (Chronic Pulmonary Obstructive Disease). This condition is characterised by early lactate accumulation during submaximal exercise. The obvious inference to make here is that this is due to an increased reliance on glycolytic metabolism and therefore lactate production. However, as it has been shown that lactate accumulation is the result of the balance between production and clearance, it can be suggested that the premature rise in blood lactate may be the result of impinged clearance (Maltais et al, 1996; Maltais, 1998). Furthermore, findings from studies which use patients with conditions which alter the physiological state cannot necessarily be translated to an athletic population due to the secondary effects of the condition. Exercise intensity is always relative to the physical capacity of the individual. Therefore, it is accepted that a diseased population (and an untrained population for that matter) can achieve a maximal test despite working for less time and at a lower absolute work rate than an athletic population. However, it is likely that this reduced duration will determine that different metabolic responses to exercise are not taxed in the same proportions as in an athletic population.

In health, it is now well established that as well as being the major site of La production, skeletal muscle also consumes and therefore clears La through oxidation (Brooks, 1985). La can also be transported to the liver where it is used as a major gluconeogenic precursor (Bergman et al, 2000). During submaximal exercise, these mechanisms work efficiently to maintain the homeostatic balance between La production and La clearance.

As the intensity of exercise increases, the glycolytic energy systems are more strenuously taxed and this equilibrium is disturbed meaning the clearance mechanisms become overwhelmed resulting in the accumulation of La (Donovan & Brooks, 1983). This shift in substrate metabolism sees a concurrent increase in $[H^+]$ as the oxidative capacity of the cell approaches the point at which it is maximised and demand for energy exceeds the ability of the muscle to supply it through aerobic means.

It should clearly be highlighted here that this rise in $[H^+]$ does not occur as a result of increased [La] (indeed the converse is true) but rather is a product of the altered intracellular metabolic conditions. Regardless, the resulting acidosis (reduction of pH) is balanced by the bicarbonate buffering system in the following chemical reaction:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO^-_3$$

Clearly, consistent use of this acid buffering system leads to an increase in the concentration of CO_2. Consequently, the central chemoreceptors in the medulla are stimulated to augment ventilation ($\dot{V}$e) and "blow off" the excess CO_2 (Wasserman, 1987). This process is the physiological foundation that underpins the theory of the ventilatory threshold which suggests that the point at which VCO_2 increases disproportionately to VO_2 can be used as an indirect measure of the lactate threshold (Walsh & Banister).

Although it is accepted that this ventilatory breakpoint can be used as a proxy measure of the lactate threshold, to date there is no equivalent ventilatory measure of lactate clearance despite efforts to establish a link between it and Excess Post Exercise Oxygen Consumption (EPOC) (Tomlin & Wenger, 2001). Currently, the rate of lactate clearance is only measurable either invasively through blood sampling or by use of isotopic tracers (Brooks, 1985). It is evident that the practicalities of performing such assessments do not easily lend themselves to the sporting arena and therefore their application is largely unrealistic within the population who are likely to be the greatest beneficiaries.

This is particularly true of team sports with a large number of members. As a result, a measure of lactate clearance which can be generated from a protocol already frequently employed is of great interest and value. Currently, evidence exists which displays an association between the mechanisms of lactate accumulation/clearance and ventilation but much of this is based on outdated observations or in non-athletic/clinical populations.

Given that the ability of the muscle to use lactate as an oxidative substrate is intrinsically related to the aerobic capabilities of the muscle cells, it seems clear that muscle fibre type may be a powerful determinant of the rate of lactate clearance. One would assume that endurance trained athletes who have been found to possess a large proportion of type I, slow twitch muscle fibres are likely to manage lactate in a more efficient manner (Brooks, 1985) and maintain performance with high concentration of blood lactate (Hoogeveen et al, 1997). This efficiency should be manifested as a delayed lactate threshold and an increased rate of post-exercise lactate clearance. Conversely, athletes trained specifically for power competition are likely to attain higher peak blood lactate levels and a relative slower rate of utilization by virtue of the reduced oxidative capacity of the type II muscle fibres which predominate (Brooks, 1999). However, in mixed modality and intermittent sports, athletes cannot train with this degree of specificity. The nature of the sport necessitates this ambiguous training so as to engender the spectrum of physical qualities required. The issue of lactate clearance and its determinants in this population is equally as important yet less well defined than in others.

Due to the physiological processes previously mentioned, it seems entirely reasonable and plausible to suggest that athletes, with a typical pulmonary response, who attain higher rates of $\dot{V}_e$ during exercise do so at least in part due to an inability to effectively clear lactate in the muscle and in the liver. This theoretically places a greater reliance on the bicarbonate buffering system, resulting in an accentuated ventilatory response to intense exercise. Given that the sensation of breathlessness is

often the factor which determines cessation of exercise, it can be argued that this process could limit exercise capacity.

We therefore postulate that peak ventilation can be used as an indirect measure of lactate clearance in athletes trained for the demands of a specific sport. The physical demands of football will be discussed later in this review. This will allow indirect measurement of lactate clearance to be conducted simultaneously with VO_{2max} and ventilatory threshold measurements which are accessible to the majority of professional sports people and squads. Also, the data generated can be used to develop and prescribe training protocols specific to improving lactate clearance.

Literature Review – Chapter 2

2.1 Historical Overview

The history of research into lactate and its physiological significance is now approaching 150 years. Seminal work by Pasteur (1863), Hill and Lupton (1923) and Meyerhoff (1920) have provided the basis for decades of conjecture, theory, argument and counter-argument with the former lending his name to the "Pasteur Effect" (lactic acid being produced at a faster rate as a result of yeast breaking down glucose in the absence of oxygen). The investigation of Meyerhoff (1920) found that in vitro electrical stimulation of isolated frog leg muscle resulted in the muscle becoming bathed in lactate. It was assumed that this accumulation was a direct result of the anaerobic condition of the tissue. The relationship between oxygen, cellular metabolism and the rise in b[La] during and post exercise was further explored by Hill and Lupton (1920) who also concluded it to be a direct consequence of a lack of oxygen in the working muscle.

This field defining work and other early research like it created a paradigm which depicted lactate as a dead end, metabolic waste product of no significant physiologic value. A common theme to the aforementioned studies is one of anaerobiosis being the key determinant of lactate production. This theory was accepted to the extent that lactate was determined as being a major cause of fatigue during high intensity exercise and its production as being indicative of the switch from aerobic to anaerobic metabolism (Hollozsy & Fitts, 1976). The observations of a concomitant decrease in power output and muscular efficiency at high b[La] (Bogdanis et al, 1995), increased b[La] during exercise (Gass, 1981) and improved endurance performance with delayed blood La accumulation (Farrell & Costill et al, 1979) make this assertion logical though to a certain extent misplaced.

Evidently this misplaced logic is attributable to the close yet somewhat indirect relationship these factors share with complex metabolic and biochemical interactions and the difficulty in displaying causative associations between them. The early speculation on changes in b[La] during exercise can

be summarised as representing lactate as a fatiguing agent during high intensity exercise, produced as a waste product only when cellular metabolism becomes anaerobic.

2.2 Current Perspective

Significant progress has since been made in relation to the understanding of the kinetics, physiology and biochemistry of lactate during exercise and their implications for athletic performance. This has led to both rejection and alteration of some of the early hypotheses. Much of this was possible due to the extensive research of George Brooks and colleagues. Perhaps the most significant finding which deviated from original theory was the discovery that lactate is produced even under fully aerobic conditions, deeming the term "Anaerobic Threshold" (Davis, 1985) inaccurate and misleading.

This was intricately shown by Connet et al (1984) who displayed an accumulation of lactate in working muscle at intensities as low as 10% of VO_{2max} in isolated dog gracilis muscle. Consequently, it is now universally accepted that the increase in b[La] observed during exercise is not due to an increase in the anaerobic production of lactate but rather a result of an imbalance between lactate production and lactate clearance (Diamant et al, 1968; Brooks, 1985) due to an altered cellular environment during exercise.

Moreover, research has disputed the assertion that lactate itself is a limiting factor where athletic performance is concerned (Brooks, 1986, 1998; Gaesser & Brooks 1984; Gertz et al, 1988). Detailed biochemical investigation has found that while the H^+ ions formed during steady state exercise are carried by NADH to be oxidized within the mitochondria, this is not the case at higher intensities. An increased reliance on the cells glycolytic mechanisms requires sufficient availability of NAD^+ to sustain ATP production. This is made possible by pyruvate's acceptance of two H^+ ions to form lactate in a reversible redox reaction catalysed by the enzyme Lactate Dehydrogenase (LDH).

This lactate formation therefore permits further glycolytic energy production and assists in continuation of prolonged exercise. However, due to the cellular conditions necessary for this to occur, it is clear that lactate can be used as an indirect marker of fatigue and its rate of clearance as a valuable indication of recovery. It has been suggested that it is more likely that the concurrent increase in H^+ retards the regeneration of ATP (Jubrias, 2003), can inhibit PFK activity (Trivedi, 1966) and can interfere with the excitation contraction coupling process (Fitts, 2003). Recent studies have also pointed to the potentially detrimental effect of the inorganic phosphates (P_i) produced from phosphocreatine (PCr) and ATP metabolism (Westerblad et al, 2002).

The evidence confirming the value of lactate as a substrate is the foundation of the established principle of enhanced lactate clearance due to active recovery (Bangsbo et al, 1994). Activity during recovery will maintain a higher level of metabolism to allow consumption of lactate without a concurrent rise in lactate production (effectively reversing the imbalance which led to accumulation). The rate of this clearance in the post exercise period can be dictated by the intensity of the active recovery. Research by Menzies et al (2010) has suggested that the optimal intensity for this recovery period is comparable to that at which the lactate threshold occurs. This is verifies the view that lactate itself is not responsible for the inability to maintain prolonged high intensity exercise. Despite this evidence, the notion that lactate is the cause of fatigue during exercise and even Delayed Onset Muscle Soreness (DOMS) is still one that is perpetuated within many sporting circles.

2.3 Physiological Demands of Football and Relevance of Lactate

The physical demands of football are determined by the regular supra maximal, dynamic intervals of activity interspersed amongst periods of relatively low level, aerobic activity (Bangsbo, 1994). The investigation by Bangsbo et al (2006) succinctly reviews the nature of these demands. Up to 18% of the distance covered during a game is done so at what is considered to be high intensity (Ekblom, 1986; Castagna et al, 2007; D'Ottavio, 2001). Such high intensity sprints are required on average every 90 seconds and last for between 2-5 seconds (Hoff, 2005). At the elite level, they become more

frequent and intense without as significant a performance decrement in the latter stages of competition (Wisloff et al, 1998; Stolen et al, 2005; Bunc et al, 2001). In physiological terms, this can be interpreted as periods of lactate accumulation followed by periods of recovery.

It has been found that lactate levels amongst footballers increase to around 7-8 $mmol.l^{-1}$ during the course of a game (MaClaren et al, 1987; Ekblom, 1986), reducing to around 4 $mmol.l^{-1}$ during the low activity periods of competition (Castagna et al, 2007). This is still significantly elevated from resting concentrations, suggesting that the frequency and possibly intensity of anaerobic bouts of exercise do not allow for total recovery. This will ultimately compromise athletic performance and these high intensity efforts will become shorter and of less power. Clearly, enhanced clearance of lactate is highly advantageous in improving athletic performance in football as measured by lactate clearance.

Due to nature of these requirements, the consensus is that it is not necessary for footballers to show an endurance capacity akin to that of an endurance trained athlete with "normal" VO_{2max} being in the region of 63-65 $ml.Kg.min^{-1}$ (Edwards et al, 2003; Wisloff et al, 2004). Typically, aerobic conditioning of football players is achieved through the use of 4x4 minute runs at 90-95% of maximal heart rate as this has been found to be an economic method to enhance VO_{2max} (McMillan & Helgerud et al, 2005). However, we must acknowledge that this improvement is a consequence of central adaptations, with the larger stroke volume allowing for increases in cardiac output (Hoff & Helgerud, 2004). A more sport specific approach is also frequently taken using small sided games to marry physical and technical elements of training. Reilly and White (2005) acknowledged the validity of this method as a useful training tool using aerobic and anaerobic metabolic processes but also reported no significant changes in peak lactate after all out exercise after a 6 week aerobic or small sided game training program. This has profound implications with regard to lactate clearance as this combination of training stimuli may be insufficient or inappropriate to engender the cellular oxidative profile known to be conducive to the removal of lactate.

Significantly, the investigation by McMillan and colleagues (2005) used elite youth soccer players as subjects who were, on average, the same age as the population in the present study (around 17 years old). This is the age at which the majority of elite youth players will make the transition from part time to full time training and so the physical demand placed on them increases greatly. While operating as part-time youth players, each player trained on average 3 days per week with focus very much on technical development. Upon their entry to full-time training, this increased to a minimum of 4 high intensity field based sessions allied to a structured lower body and upper body weights program and one game per week.

It has been suggested, both anecdotally and in the literature, that this enlarged training load can cause an increased incidence of musculoskeletal injury (Deehan et al, 2007). This fact was acknowledged in an English Football Association audit into injuries in their academies where incidence of injury, particularly those non-contact in nature, increased sharply as players became full time professionals (Price et al, 2004). Even without the sudden increase in physical load, the stage of biological maturity each player is experiencing can influence the pattern, severity and frequency of injury (Le Gall et al, 2006).

Provided the training stimulus is sufficient, a number of physiological adaptations will occur. In the intervention by McMillan et al (2005), this adaptation was an improvement in VO_{2max}, however, the same positive results have also been found for interventions centered on improving power and agility (Thomas et al, 2009),strength (Wisloff et al, 1998) and speed (Wisloff et al, 1998; 2004). Consequently, care must be taken to ensure that each athlete has been subjected to the physiological and metabolic stresses of full time football training for an appropriate length of time for inferences to be made regarding elite youth footballers as a population. Appropriately, subjects in the present investigation were subject to at least 6 months of such training before testing took place to ensure accurate testing of the hypothesis was possible.

Pertinent questions are raised here in relation to the training and testing of professional football players. For instance, given its evident importance, how can lactate clearance be measured when a large squad must be tested? Also, is the training currently undertaken by football players sufficient for the demands of the sport? These questions are applicable across a myriad of mixed modality sports where the physiological stresses presented are varied and physiological aptitude must reflect this. The testing protocol in this study was designed in order to rapidly generate fatigue in each athlete to allow analysis of the recovery period. It is noted that the demands of incremental, prolonged, high intensity exercise to volitional exhaustion on a gradient are not necessarily comparable to that of the sport. However, the fact that the variables of particular interest were the post-exercise dynamics of ventilation and blood lactate determined that the mode of testing was entirely sufficient.

2.4 Lactate Clearance in the Muscle

As well as being the site of lactate production, the muscle is largest consumer of lactate during exercise and therefore fundamental to lactate clearance. The principal mechanism for lactate clearance is through oxidation. This process accounts for around 50% of total clearance at rest and between 70% and 75% during exercise (Brooks, 2009). In this process, lactate produced in predominantly glycolytic (type II) muscle fibres is used as a fuel source by proximal oxidative (type I) fibres.

Lactate is also found to be the primary fuel source for cardio myocytes during exercise (Gertz et al, 1988). The importance of lactate as a fuel source is shown by the fact that, during exercise, blood lactate flux may exceed that of blood glucose flux (Bergman, 2000). In aerobic conditions, lactate is oxidized to form pyruvate to enter to the Cori Cycle with the reverse redox reaction occurring in an environment of insufficient oxygen, both of which are catalysed by LDH (Hochachka, 1980).

This process of distribution of lactate to oxidative tissues has been termed the “Cell-Cell Lactate Shuttle” (Brooks, 1985). For this to be the case, a transporting mechanism must be in place. This was

established through the discovery of monocarboxylate transporters (MCT) (Juel & Halestrap, 1999). The isoforms MCT1 and MCT4 are found in skeletal muscle to allow lactate to cross extra and intracellular membranes and are found to be upregulated as a result of training (Baker et al 1998; Pilegard et al, 1999). Subsequent research has found that lactate can be oxidized within the mitochondria even in the absence of extracellular conversion to pyruvate, giving rise to the "Intracellular Lactate Shuttle" (Brooks, 1998; 1999b). Such findings are supported by evidence of lactate oxidation in isolated mitochondria made possible by an intracellular pool of LDH and the presence of MCT1 (Dubouchaud, 2000; Hashimoto, 2008). The work of the mitochondria with regard to the shuttling of lactate is essential to maintain the necessary gradients required for lactate flux.

2.5 Lactate Clearance in the Liver:

A supplementary method of lactate clearance occurs in the liver. This accounts for around <20% of the clearance of lactate during exercise (Brooks, 1998). Despite this comparatively low contribution, it has been established that lactate is the major gluconeogenic precursor (Donovan & Brooks, 1983). This is made possible by the presence of a hepatic pool of LDH which shows a high capacity for lactate oxidation (Kline, 1986). The resulting pyruvate can then enter the "Cori Cycle" to be used either to form glucose for immediate energy metabolism or for glycogenesis to replenish the hepatic carbohydrate stores. The glucose generated can be transported back to the working muscle where it can be used in glycolysis by glycolytic fibres to allow a continual cycle which lends credence to the paradigm of lactate as a metabolic intermediate as opposed to a waste product or fatiguing agent. The close relationship between lactate production/clearance and substrate metabolism has been shown effectively (Brooks, 1988; Jansson, 1982).

From this evidence it seems clear that lactate is unlikely to be a limiting factor in athletic performance. Training can allow for more efficient transport of lactate across the membrane into the blood due to the activity of sarcolemmal membrane proteins (Mcdermott & Bonen, 1993). Also observed has been an increased hepatic capacity for gluconeogenesis (Stallknecht et al, 1998). The

superior cardiac output and capillarisation observed as a result of endurance training may allow maintenance of an adequate blood flow to the liver and therefore sustain a higher rate of liver lactate clearance for a given work rate than an untrained subject. This enhanced distribution of blood is likely to give rise to a more efficient lactate/glucose turnover due to the reduced transit time required and can possibly be viewed as the link between the two mechanisms of clearance.

2.6 Measurement of Lactate Clearance

The most commonly applied method of profiling lactate clearance, particularly in the field, is through the use of capillary blood samples at specific time points. Samples are generally taken from the finger tip or ear lobe and can be analysed immediately using, for instance, an accutrend device (Perez et al, 2008; Baldari et al 2009) or preserved to be analysed retrospectively using devices such as the GM7 lactate analyser (Someren et al, 2005).

The b[La]measured can be plotted against time during the recovery period to show the behaviour of lactate post exercise. It has been suggested by some studies that these samples can be taken at regular intervals for15 minutes post exercise to allow for accurate application of mathematical modelling of lactate clearance (Gharbi et al, 2008). Although this time period is frequently extended and it may be that its popularity is a product of convenience rather than accuracy.

Yeh et al (1983) acknowledged the difficulty in making inferences using this invasive measure. However, Williams et al (1992) recommended the use of capillary blood samples due to their reflection of arterial concentration while recognising the importance of sampling site .The limitation of this method of measuring lactate clearance is clear. Despite its invasive nature, this is always an indirect measure as it is an indicator of the balance between lactate production and clearance rather than a direct measure of the removal of lactate in the working muscle. Clearance of lactate in the blood is unlikely to be equal to that in the muscle due to the intracellular mechanisms at work (Brooks, 1998).

A more complex method of measurement is now in place and used increasingly within a laboratory setting. This involves the use of isotopically labelled tracers to follow the movement of lactate over a period of time. It is proposed that the rate of disappearance of lactate can be determined by dividing the rate of tracer infusion by the plateau lactate specific radioactivity in the blood (Freminet et al, 1984). Studies have confirmed this method to be accurate during muscular exercise, even when glycolytic flux is greatly increased (Stanley & Brooks, 1987).

The direct analysis of lactate movement is highly advantageous and provides detail of lactate clearance time course and the kinetics of the process. This is shown by the fact that all of the modern day, field-defining research into the intricacies of lactate clearance have utilized this method (Brooks et al, 1985, 1986, 1998, 1999). A succinct review of the use of isotopic tracers is provided by Stanley and Lehman (1988) who confirm its accuracy and reproducibility within a laboratory setting. However, it is clear that this is not transferable to a sporting environment, particularly in team sports. A combination of the lack of availability of isotopic tracers, the cost involved and the time required for their use make them highly impractical in this population. Consequently its accuracy is compromised by the reality of use in the field.

The balance of lactate clearance and production can be and is measured by a Maximal Lactate Steady State test. This is defined as the highest b[La] and work rate reached by an athlete during the test without a progressive accumulation of lactate. Protocol for such an assessment is well described by Palmer et al (1999) and built on by Kuphal (2004). Great insight can be gained here as the ability of the athlete to produce and use lactate effectively can determine endurance running performance and is indicative of the metabolic activity of the muscle. However, the nature of testing in football determines that the advantages of this test are outweighed by the practicalities and logistics of conducting it. It requires athletes to be profiled in depth and usually requires more than one testing period. With a large squad of players, this is unfeasible. Despite the fact that the apparatus required

for such a protocol may prove to be financially viable for football clubs, it does not provide the scope to test other variables in the same manner as a ventilatory analyser and is therefore not as economic.

2.7 The Relationship Between Lactate Clearance and Ventilatory Measures:

The work of the early researchers has intrinsically linked lactate production and metabolism with ventilatory processes. When Margaria et al (1933) evaluated the biphasic construct of VO_2 in recovery from exercise, they hypothesised that the initial fast decline in VO_2 (termed the "alactacid mechanism") resulted from the restoration of phosphogens to high energy phosphates while the secondary slow component ("lactacid mechanism") reflected lactate clearance. It has since been found that this is too simplistic a model due to the number of variables and metabolic processes involved (Gaesser & Brooks, 1984).

Ventilatory variables are accepted as an indirect measure to determine when lactate clearance and buffering mechanisms have become insufficient during intense exercise at a point known as the "respiratory compensation point" (the point at which Ve and VCO_2 lose linearity). The physiological processes which determine Ve and lactate clearance mean that they are inextricably related. The dynamic and transient interaction of these processes makes resolving which factors are most influential extremely difficult (Meyer, 2004).

Classically, hyperventilation was attributed to the dissociation of lactic acid to lactate and H^+, causing acidosis and a compensatory ventilatory response. Although logical, there is no strong foundation to this theory as lactate does not cause but actually diminishes acidosis caused by an alteration in the metabolic behaviour of the cell (Rogbergs et al, 2004). The complexity of the relationship is exemplified by the finding that while endurance training enhances both the oxidative mechanisms of lactate clearance and VO_2, these improvements are disproportionate to each other (Holden et al,

1995). This is further evidence of the intricate linking of different physiological processes connecting lactate clearance and ventilation.

As previously mentioned, some studies have investigated the response to different intensities of exercise in patients suffering from Mcardle's syndrome. This is of significance due to the enzymatic deficiency elicited by this condition with one of the effects being an inability to produce lactate. It has been shown that these subjects still show a marked hyperventilation during exercise (Hagberg et al, 1982; Patterson et al, 1990). Such findings suggest that ventilation during exercise is controlled by factors out with lactate, H^+ and pH. While this illustrates the complexity of the relationship well, it is not necessarily applicable to trained individuals with more efficient and sensitive ventilatory responses and also assumes normal chemoreceptor behaviour. Assertions have also been made to suggest that ventilation during exercise is more closely related to plasma [K^+] than [La] (McMurray & Tennan, 2010; Paterson et al, 1990). However, more attention should be paid to ventilation at intensities approaching and beyond 100% of VO_{2max} rather than focussing on the curve of ventilation throughout exercise. Furthermore, these assumptions have worked almost exclusively on the premise that b[La] influences ventilation during exercise by inducing "lactic acidosis". As well as incorrectly inferring a casual relationship, this does not specifically take into account the mechanisms of lactate clearance and how they can potentially alter ventilation.

An element of clarity has been provided by research into the exercise tolerance in COPD patients. It has been found that this population show an early accumulation of lactate during exercise (Stein et al, 1982), not due to a decreased oxygen delivery (Maltais, 1998) along with reduced oxidative enzyme activity (Maltais et al, 1996). Furthermore, exercise training is found to proportionally decrease b[La] and Ve during submaximal exercise without a change in FEV_1 i.e. an improvement at a muscular level (Casaburi & Wasserman et al, 1991). The significance of this finding lies in the fact that it recognises

a link between known intermediates of lactate clearance and the pulmonary state and supports the need for further detailed research to determine the nature of this link.

Methods

3.1 Subjects

The cohort consisted of 14 male subjects, as determined by a power calculation, of mean age 17±0.7 years, mean height 182±7 cm (*Seca Leicester Portable Height Measure)*, mean body mass 75±8 Kg (*Seca 888 Compact Digital Floor Scale, Canterbury, England)* and mean body fat 9.9% (*Harpenden Skin Fold Calipers, Sussex, England)*. Body fat was measured by an ISAK trained investigator using the Jackson/Pollock method All subjects were well trained, professional, youth football players who were recruited due to their status as members of the academy of a Scottish Premier League football club. A proportion of the subjects were entering their first year of full time football training. Consequently, the cohort had not received a training volume to engender sufficient physiological adaptations. As such, testing was delayed for a period of at least 6 months to allow subjects to make the appropriate adaptations to the increased workload and physical demands. Participants were provided with an information sheet detailing the protocol before written consent was provided. It was made clear to each participant that they were not obliged to complete the protocol and could withdraw without reason at any point. Under the terms of each individual's contract, all subjects have undergone the medical screening process of the Scottish Football Association including an echocardiogram and ECG and therefore were determined to be suitable for the protocol. The study protocol was approved by the College of Medical, Veterinary, and Life Science ethics committee of the University of Glasgow.

Table 3.1 illustrates the age and morphological characteristics of each of the subjects tested. This shows the natural heterogeneity of the sample, typical of that found in team sports. Mean body mass was 75 Kg from a range of 62-83 Kg and mean body fat was 9.9%.

Table 3.1: The age and morphology of subjects within the cohort

	Age	Height (cm)	Body Mass (Kg)	Body Fat (%)
Subject 1	18	180	74	9.2
Subject 2	17	191.2	83	7.9
Subject 3	17	183	69	10.2
Subject 4	17	170.2	71	11.3
Subject 5	17	181	92	8.2
Subject 6	17	187.8	77	8.9
Subject 7	17	186.4	80	11.5
Subject 8	18	176	70	8.6
Subject 9	16	183	74	11.9
Subject 10	16	168	62	9.2
Subject 11	16	177	72	9.9
Subject 12	18	195	85	12.2
Subject 13	16	187	66	8.8
Subject 14	17	188.5	73	10.4

3.2 Protocol

Prior to commencement of the protocol, capillary blood samples were taken via thumb prick and immediately analysed for resting blood lactate and glucose concentrations prior to any exercise using an Accutrend plus device (Roche Diagnostics, Germany). Participants only completed the protocol if resting measurements fell within the expected parameters. Subjects did not attend the testing session in a fasted state so as to more closely replicate pre-training, pre-competition and pre-testing conditions, however, resting blood glucose measures did not deviate from expected values. Subjects then completed a 2 minute treadmill warm up at a set speed of 6 Kmh^{-1} while wearing respired gas analysis apparatus. This was deemed to be the only familiarisation necessary as despite the inexperience of the cohort they have been subject to exercise testing in the form of beep tests and MAS runs from the age of 12 and frequently experience high exercise stress. Heart rate and ventilatory measures were monitored during this period to ensure their stabilisation. Following this period, a maximal treadmill ramp test was undertaken until termination at volitional exhaustion. The protocol used was a Taylor Protocol (Taylor et al, 1955) with treadmill speed being set at a constant speed of 10 Kmh^{-1} and gradient increments of 2% every 2 minutes after commencement at a 0% gradient. Throughout the protocol, $\dot{V}e$, $\dot{V}O_2$, $\dot{V}CO_2$ and RER were measured by respired gas analysis with a 10 second average (Astorino et al, 2000) using a Cortex Metamax II (CORTEX Biophysik, GmbH, Germany) appliance which uses a dynamic mixing chamber for analysis. Heart rate was monitored continuously by Polar Team System and recorded along with Rating of Perceived Exertion (RPE) at the end of each increment. RPE was determined using the established Borg Scale. VO_{2peak} was determined by calculating the mean VO_2 over the final 30 seconds of exercise prior to cessation. The ventilatory threshold breakpoint was calculated visually from the graphs generated by the metamax software. A period of active recovery was completed following cessation of the maximal test at a predetermined speed of 5 Kmh^{-1} on a gradient of 1%. During the active recovery, capillary blood samples were taken via thumb prick at exhaustion and 1 minute, 3 minutes, 5 minutes and 15 minutes post-exercise and immediately analysed for blood La concentrations. $\dot{V}e$, $\dot{V}O_2$, $\dot{V}CO_2$ and RER were continuously recorded throughout this period also. The length of the recovery period was

determined as 15 minutes as research suggests this is the time course for the slow phase of post exercise oxygen consumption (Bilat et al, 2002 ; Margaria et al, 1933) and sufficient time for application of a mathematical model to determine the rate of lactate clearance (Gharbi et al, 2008).

3.3 Physiological Characterisation of Athletes

Each individual was subject to a battery of tests throughout the course of the season, as is standard with all football clubs. This allowed access to a range of data that was used to compliment the research conducted within the study and to characterise each subject with regard to physiological characteristics and therefore ascertain whether any relationship exists between lactate clearance and specific physiological attributes. Maximal Aerobic Speed (MAS) was used as an indirect measure of the aerobic capacity of subjects (Chtara et al, 2005), counter movement jump height as a proxy measure of power (Markovic, 2007) and speed over 5, 10 and 20 metres was. As a consequence it is possible to gain an insight into these athletes and from this determine whether their physiological qualities show an association with the ability to clear lactate. Maximal Aerobic Speed (MAS) was calculated using a modified Montreal Track Running test (Baker, 2011). Counter movement jump height was calculated using the Just Jump vertical jump mat (Probiotics inc. Huntsville, AL, USA). 5, 10 and 20 metre sprint time was recorded using Brower Timing System Test Centre (TC) Timing System Kit (Brower Timing Systems, Utah, USA).

3.4 Analysis of blood lactate

Blood lactate concentrations (b[La]) at the aforementioned recovery time points were plotted to show the behaviour of post exercise La clearance. The homogeneity of the protocol allowed for analysis of peak b[La] ($b[La]_{peak}$) and the half time of b[La] removal (t ½ b[La]). The half time of b[La] removal is defined as the time that it takes the b[La] to return to half of the delta value between $b[La]_{peak}$ post exercise and the observed resting b[La] measured prior to the test. This was calculated using a linear

regression fit between b[La] and the post exercise recovery time (McLellan and Skinner, 1982). The proposed biphasic nature of La removal (Oyono-Enguelle et al, 1993) decreed that every curve be fitted using the following biexponential equation (Freund and Gendry, 1978):

$$\text{Eq 1: } [La](t) = [La]0 + A_1(1-e^{-\gamma 1.t}) + A_2\,(1--e^{-\gamma 2.t})$$

In this model:

- [La](t) and [La]0 are the b[La] (mmol.l-1) after time (t) post exercise and at commencement of recovery respectively.
- A_1 and A_2 (mmol.l-1) are the amplitudes of the two exponential components.
- γ_1 and γ_2 are the time constants.
- These values were determined using the software package *Microsoft Origin5.0.*

3.5 Apparatus

Both blood lactate and glucose concentrations were obtained using an *Accutrend® Plus* device (Roche Dianostics, Germany). This device has been validated for use in both a sporting and clinical environment and was therefore deemed to meet the requirements of this study (Perez et al, 2008; Baldari et al 2009). This was tested prior to the protocol by measurement of lactate on the same subject, at the same time and under the same conditions over a 5 day period which gave a coefficient of variance of 0.15% as shown in *table 3.5*. Blood lactate concentration over this 5 day period was, in chronological order, 1.38 $mmoll^{-1}$, 1.63 $mmoll^{-1}$, 2.1 $mmoll^{-1}$, 1.72 $mmoll^{-1}$ and 1.69 $mmoll^{-1}$. Ventilatory parameters were measured through a *Cortex Metamax® II* (CORTEX Biophysik GmbH, Germany). This method of respired gas analysis has been found to be reliable during exercise (Larsson et al, 2004). Heart rate was recorded using Polar Team System equipment (Polar Electro UK, Warwick, UK). Treadmills used to complete each test were *Technogym 700 IFI (*Technogym UK, Bracknell, Berkshire). All tests were completed within the training facilities of Celtic Football Club.

3.6 Statistical Analysis

All results are expressed as mean ± SD. The power calculation (see Appendix G) determined that a minimum of 13 participants were required to complete the protocol to show any statistical significance in the relationship between lactate clearance and the ventilatory parameters measured. That is, the probability is 90 percent that the study will detect a relationship between the independent and the dependent variables at a two-sided 0.05 significance level, if the true change in the dependent variables is 17.3 units per unit change in the independent variable. Pearson's Correlation coefficient was used to determine the nature of the relationship between the variables as the interest of the study is to determine the strength of the relationship between two quantative, continuous variables. Significance was determined as $p < 0.05$.

Table 3.5: Coefficient of variance

Day	B[La] ($mmoll^{-1}$)
1	1.38
2	1.63
3	2.1
4	1.72
5	1.69
SD	0.258
Mean	1.704
COV	0.151

Results

4.1 Measured resting physiological parameters

Table 4.1 represents the mean values of the variables of interest at rest to distinguish the variability of the cohort and their pre-testing (and therefore pre-competition) physiological state. Resting blood lactate and glucose concentration was 1.65(±0.65) and 4.51(±0.8) mmol.l^{-1} respectively. Mean resting RER was 0.77 (±0.08). Minute ventilation at rest was 3.68 (± 1.06) l.min^{-1}. Baseline VO_2 and VCO_2 was measured as 0.12 (±0.04) l.min^{-1} and 0.09 (±0.03) l.min^{-1} respectively. All mean resting fall within the expected parameters for the sample.

4.2 Measured peak physiological parameters

Table 4.2 shows the mean ventilatory and blood sample values measured at peak exercise. Blood lactate concentration increased to a maximum of 8.96(± 3.3) mmol.l^{-1}. The lactate threshold calculated by ventilatory breakpoint occurred, on average, at 62.5% of VO_{2peak}. RER reached 1.31(± 0.2) with all subjects comfortably exceeding the 1.1 generally accepted as being needed to indicate a maximal test. At the cessation of exercise, the mean minute ventilation was 148.65 (±23.52) l.min^{-1}. At peak exercise VO_2 and VCO_2 were found to be 4.7(± 0.7) (58.8± 9.9 ml.Kg.min^{-1}) and 4.97(± 0.97) l.min^{-1} respectively.

Table 4.1: Mean (±SD) value of ventilatory and blood sample parameters at rest

Rest	
VO_2 ($l.min^{-1}$)	0.12(±0.04)
VO_2 ($ml.Kg.min^{-1}$)	1.58(±0.56)
VCO_2 ($l.min^{-1}$)	0.09(±0.03)
RER	0.77(±0.1)
Ve ($l.min^{-1}$)	3.68(±1.1)
b[La] ($mmol.l^{-1}$)	1.65(±0.7)
b[Glu] ($mmol.l^{-1}$)	4.51(±0.8)

Table 4.2: Mean (±SD) value of ventilatory and blood sample parameters at peak exercise.

Peak	
VO_2 ($ml.Kg.min^{-1}$)	58.8(±9.9)
VO_2 ($l.min^{-1}$)	4.6(±0.7)
VCO_2 ($l.min^{-1}$)	4.97(±0.9)
RER	1.31(±0.19)
Ve ($l.min^{-1}$)	148.65(±23.5)
b[La] ($mmol.l^{-1}$)	8.96(±3.25)
LT (% of VO_2)	62.5(±8.7)

4.3 Post exercise blood lactate

Figure 4.3 shows mean post exercise lactate profile for the cohort. Mean peak b[La] was 8.96 (±3.25) mmol.l^{-1} , measured upon cessation of exercise (*table 4.2).* After 1, 3 and 5 minutes b[La] was 7.04 (±3.79) mmol.l^{-1} , 5.23 (±2.61) mmol.l^{-1} and 4.11 (±2.66) mmol.l^{-1} respectively before decreasing to a minimum of 3.2 (±1.47) mmol.l^{-1} 15 minutes post exercise. This was 1.46 mmol.l^{-1} greater than the mean resting b[La] of 1.74 (±0.74) mmol.l^{-1} (*table 4.1 & figure 4.3).*The large standard deviation is again indicative of the physiological variability in the cohort.

The mean T ½ b[La] was 11.7 (± 5.7) minutes. Lactate clearance was fitted using both a linear regression model and the aforementioned biexponential decay equation. Significantly, the biexponential equation showed only one time course for the removal of lactate i.e. a uniphasic curve with γ1 and γ2 being identical (3.49582).This was also true for each subject as, despite γ1 and γ2 varying between subjects, no individual post exercise lactate profile displayed two components with both time constants remaining indistinguishable for each. Consequently, T ½ b[La] as fitted using linear regression analysis accurately describes post exercise lactate clearance in this sample as there is no deviation from the initial time course.

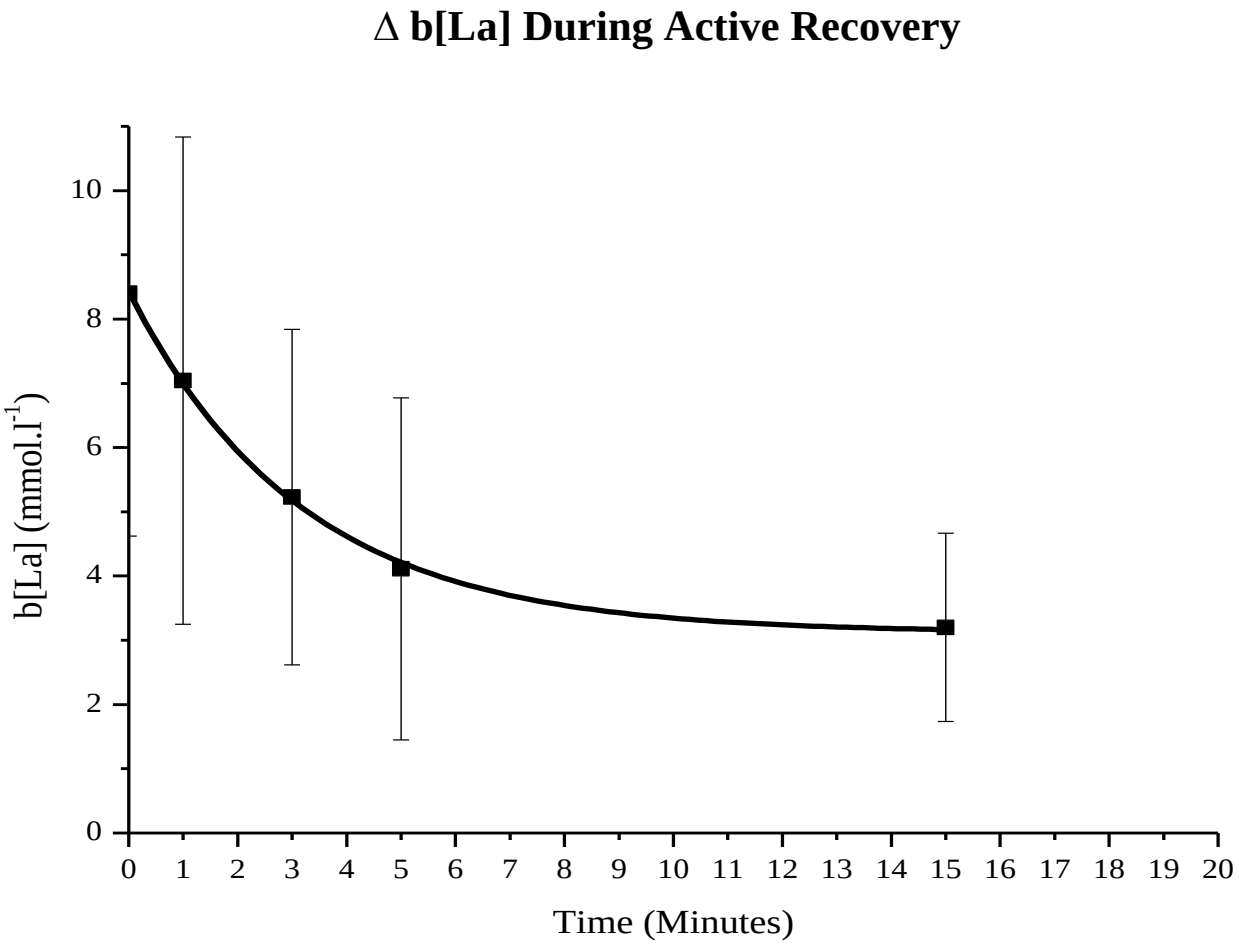

Figure 4.3: Mean change in b[La] post exercise

4.4 Relationship between b[La] and ventilatory measures

The ability to remove lactate after exhaustive exercise was strongly and positively correlated with peak minute ventilation (r = 0.65). The unambiguous nature of this relationship is shown graphically in *figure 4.4.1* with subjects who reach higher levels of pulmonary ventilation during maximal exercise showing a lengthened half-time of blood lactate clearance.

At peak exercise, minute ventilation and RER display only a weak association (r = 0.3), the behaviour of which is evident in *figure 4.4.2*. From this data, the variability of Ve_{peak} is evident with a substantial deviation from the mean (± 23.5). There is a degree of disparity with regard to RER_{peak}, although not to an equivalent extent (±0.2).

There was a moderate correlation observed between VO_{2peak} and T ½ b[La] (*figure 4.4.3*, r = 0.48). The trend in *figure 4.4.3* insinuates that subjects in this sample with a higher aerobic capacity are likely to exhibit a prolonged time course for lactate removal.

This is reflected in the relationship between lactate clearance and time to exhaustion (*figure 4.4.4, r = 0.43).* Despite the similarity in correlation co-efficient, there is a noticeably larger deviation from positive trend with regard to the time to exhaustion (*figure 4.4.4)* when compared to that of maximal oxygen uptake (*figure 4.4.3).*

T ½ b[La] was plotted against ventilatory threshold as a pseudo-measure of how the economy of movement related to lactate removal ability. A relative variability was found in the point of lactate threshold between subjects (SD ±8.7%). There is clearly no relationship between the lactate threshold measured by v-slope and the rate of lactate clearance (*figure 4.4.5*, r = 0.07).

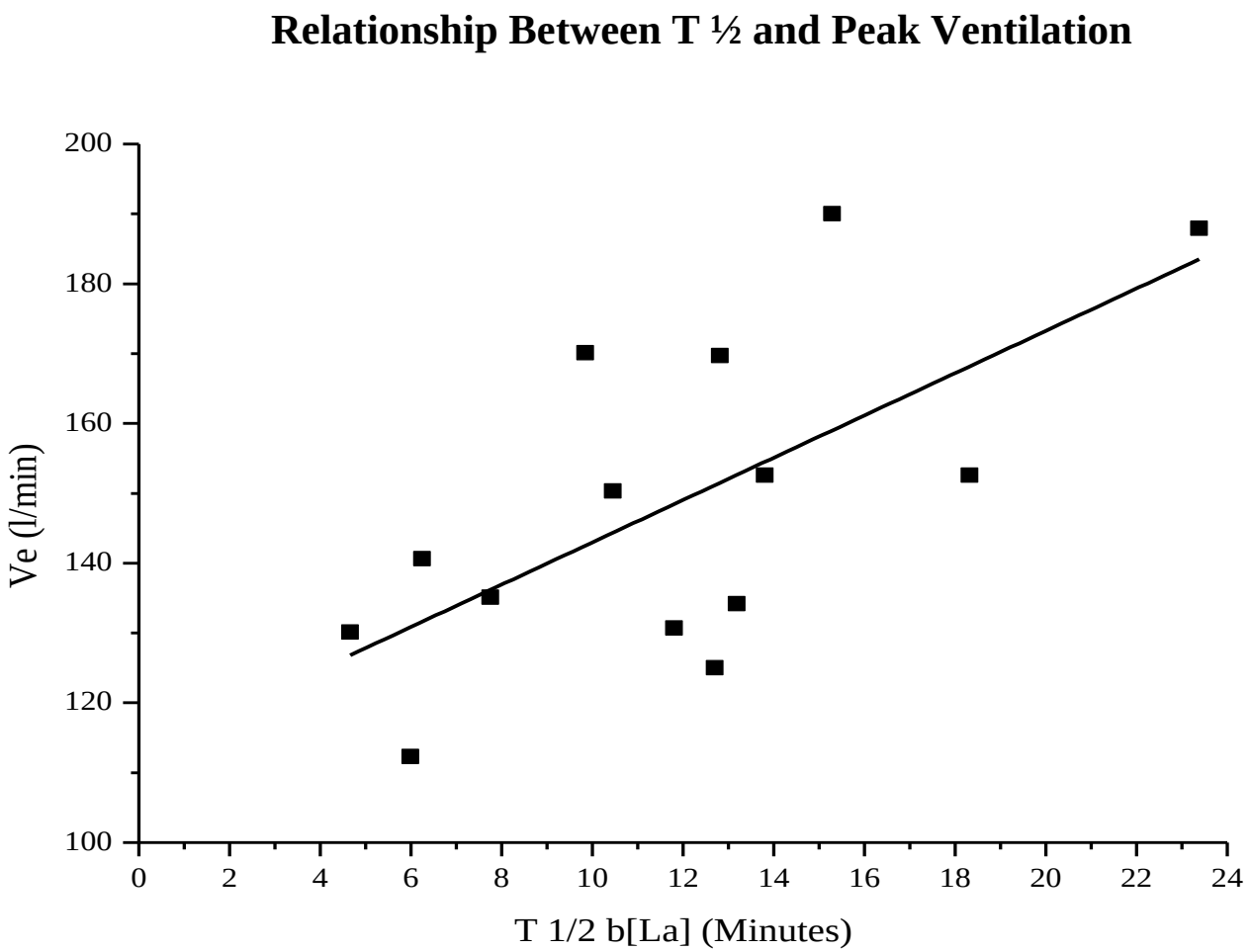

Figure 4.4.1: The relationship between T ½ b[La] and peak minute ventilation*(r = 0.65).*

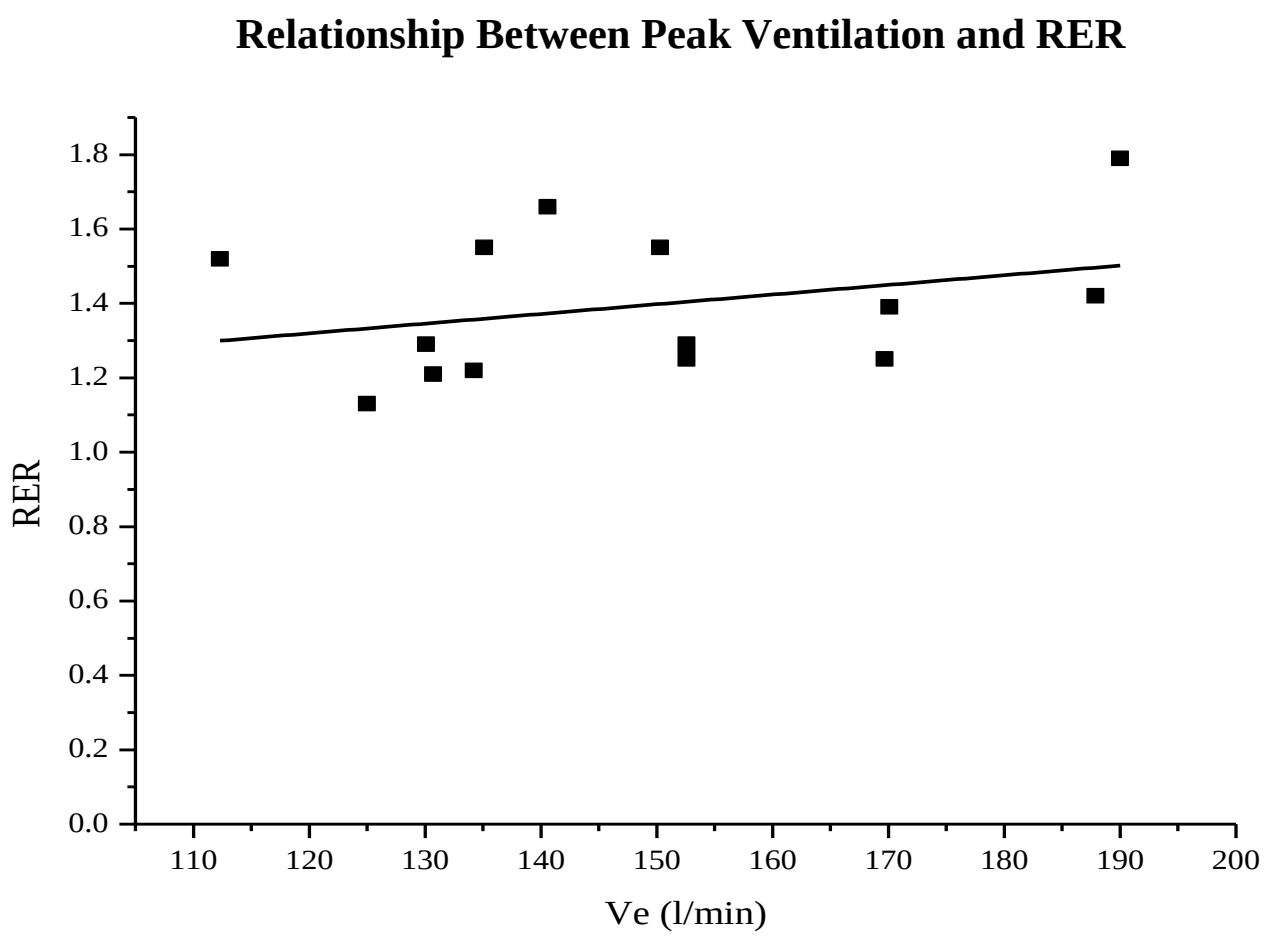

Figure 4.4.2: The loose association between RER and Ve at peak exercise. (r = 0.3)

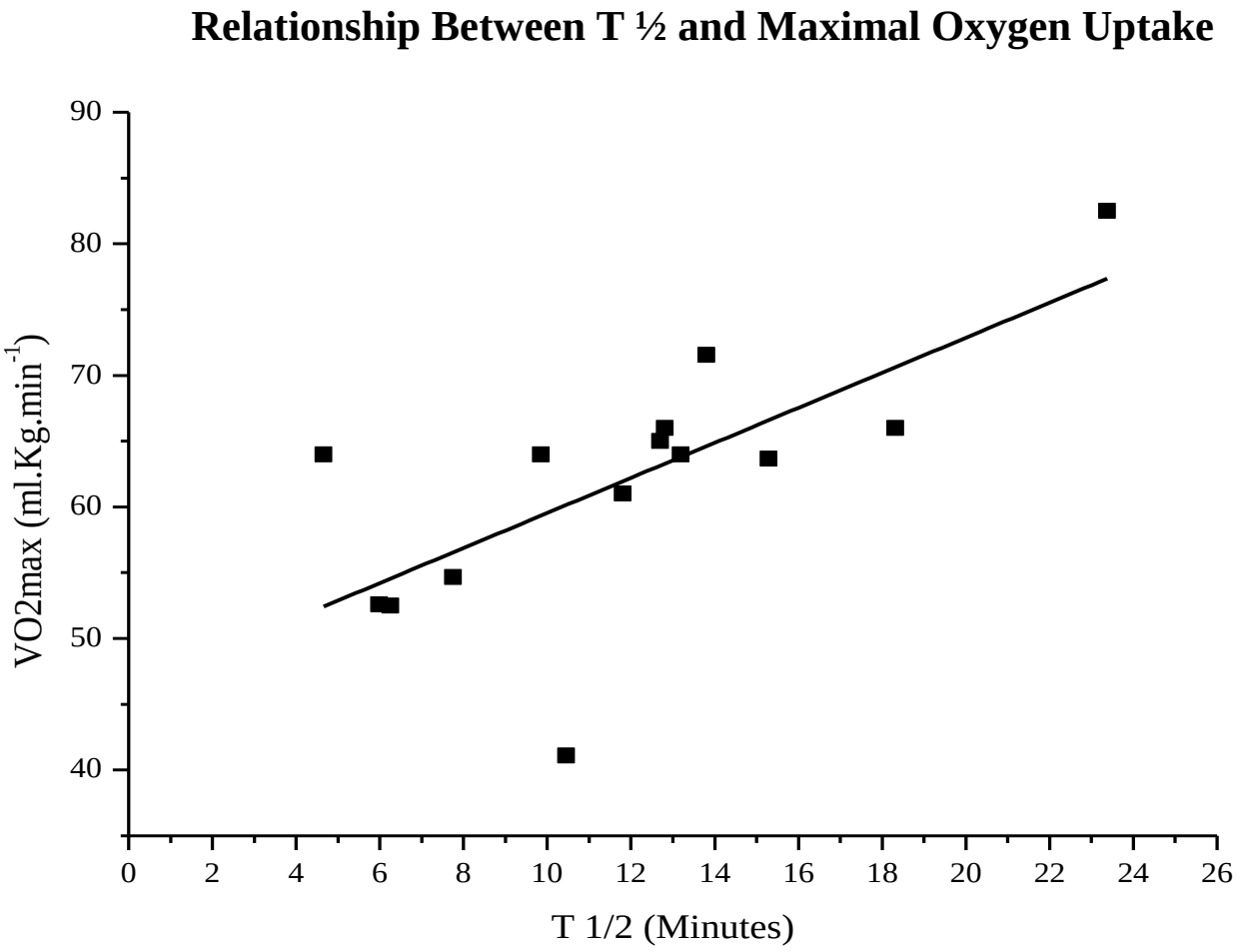

Figure 4.4.3: The relationship between T1/2 b[La] and maximal oxygen uptake *(r = 0.48)*

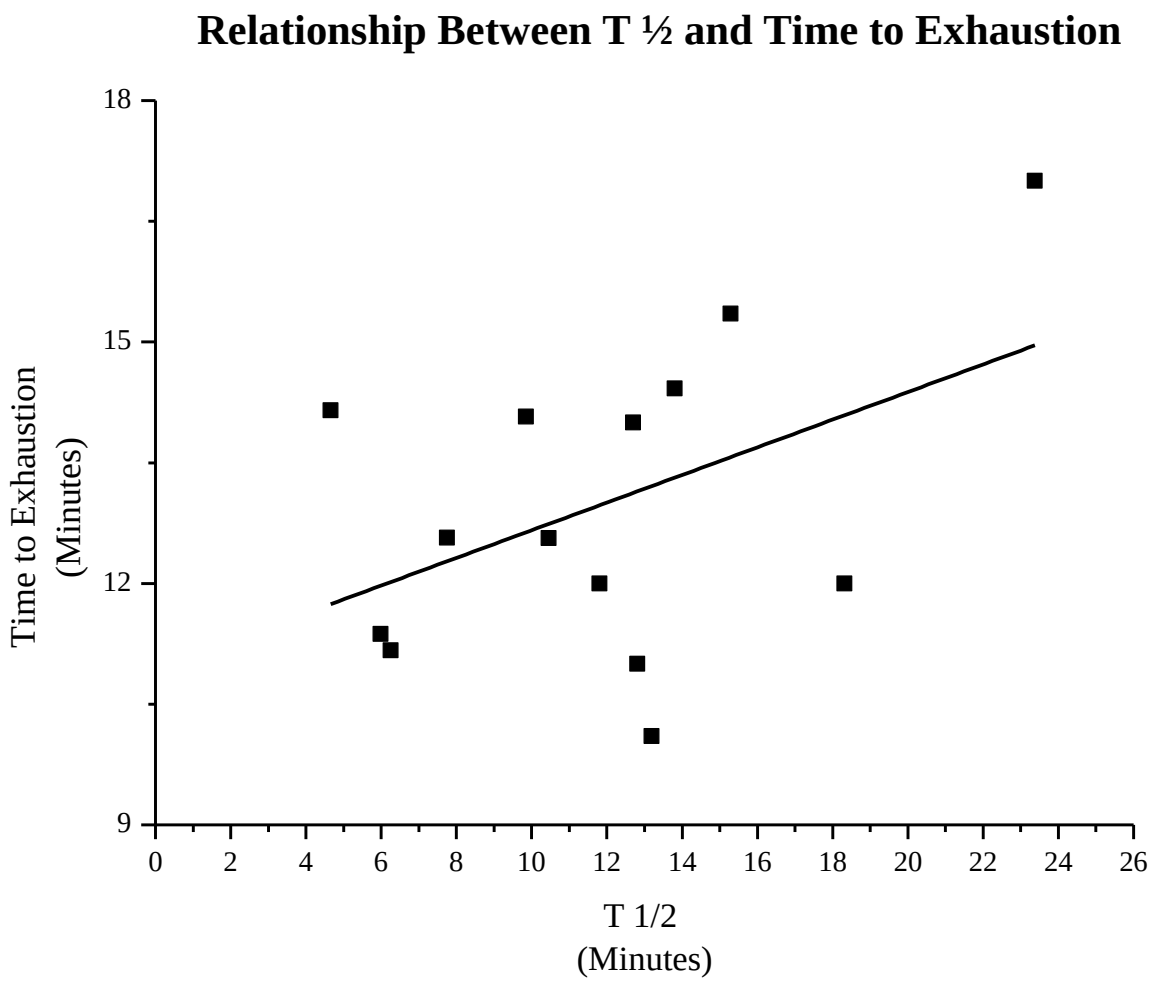

Figure 4.4.4: The relationship between length of time to exhaustion (i.e. Ve_{peak}) and T ½ b[La] (r = 0.43).

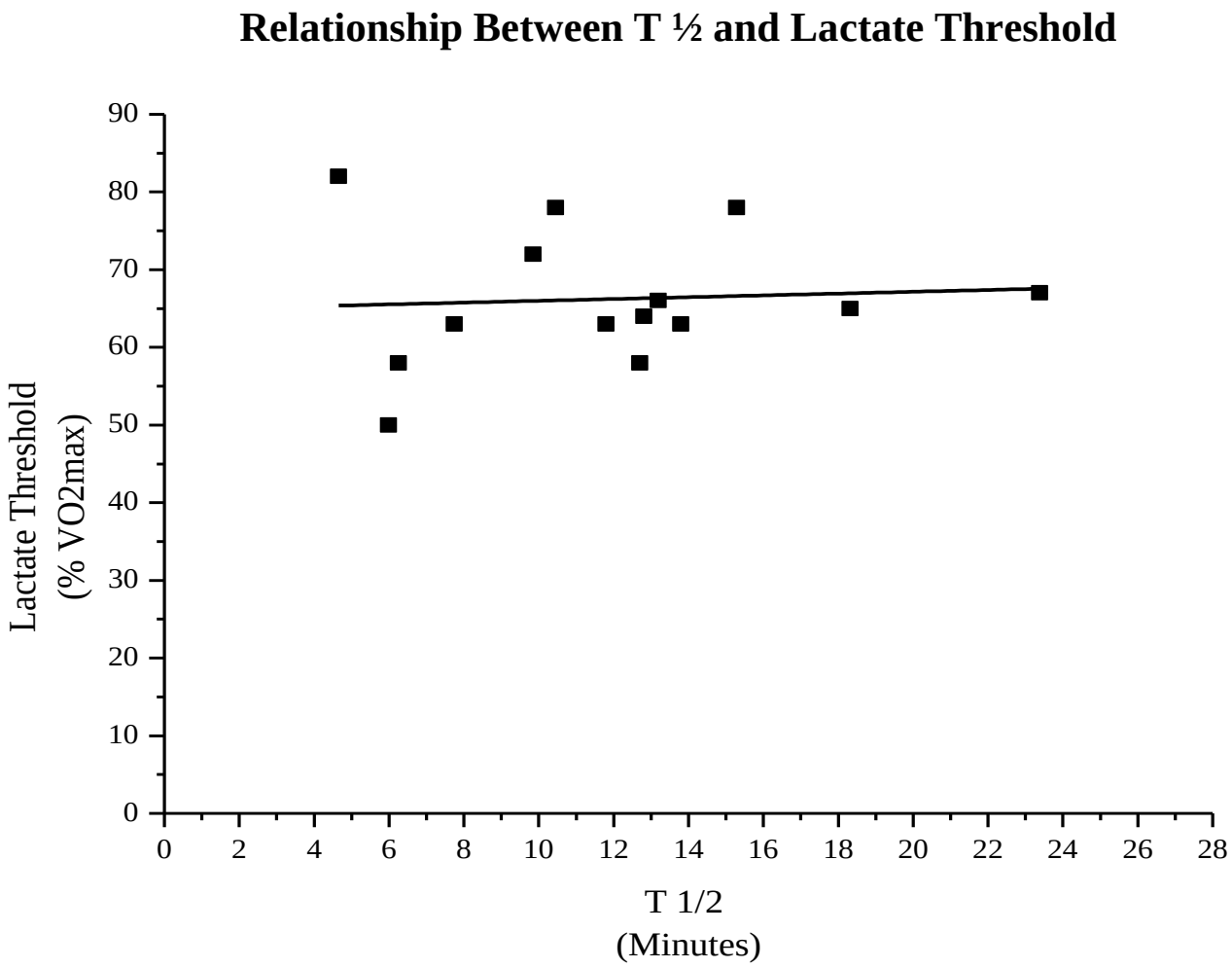

Figure 4.4.5: The relationship between the removal of blood lactate and the point of ventilatory threshold.

4.5 Physiological characterisation of cohort

The physiological measures shown in *table 4.5* characterise the cohort as athletes based on the attributes engendered through training and the relationship they share with the half time of blood lactate clearance. Overall, these results demonstrate a high level of relative lower limb strength with half of the subjects squatting more than 150% of their body weight. In some instances, this strength is translated to a more than reasonable level of power which can be indirectly measured using counter movement jump height. However, this is not exclusively the case as shown by the correlation between squat and CMJ height ($r = 0.5$). There is a significant degree of disparity in the aerobic capacity, as measured by MAS, of each subject.

Table 4.5: Individual physical characteristics from in-season testing used to determine strength, speed, power and aerobic capacity. T½ included to allow comparison.

	T ½	5m (sec)	10m (sec)	20m (sec)	Squat (%BW)	MAS (min:sec)	CMJ (cm)
Subject 1	15.29	1.14	1.84	3.06	173%	17:44	49.8
Subject 2	9.85	1.15	1.83	3.01	154%	14:24	61.0
Subject 3	4.66	1.19	1.9	3.14	136%	16:04	52.6
Subject 4	13.8	1.06	1.8	3.02	157%	14:24	59.7
Subject 5	6.25	1.03	1.77	2.95	157%	14:24	62.5
Subject 6	7.75	1.03	1.74	3	156%	17:02	54.9
Subject 7	10.45	1.1	1.78	3.02	157%	15:24	62.5
Subject 8	5.99	1.09	1.8	3.04	127%	13:30	49.0
Subject 9	11.8	1.15	1.96	3.31	142%	15:24	42.6
Subject 10	13.19	1.18	1.91	3.24	129%	16:44	45.7
Subject 11	12.7	1.22	1.98	3.26	160%	15:24	48.7
Subject 12	12.81	1.15	1.89	3.27	129%	15:00	53.5
Subject 13	18.32	1.06	1.8	3.12	136%	15:04	50.8
Subject 14	23.38	1.13	1.91	3.1	95%	17:44	48.3

$T^{1/2}$[La] = Half-time of blood lactate clearance; %BW = % of body weight; MAS = Maximal Aerobic Speed; CMJ = Counter Movement Jump.

4.6 Blood lactate clearance and measures of performance

When 5m sprint performance is used as an indicator of acceleration, it is not at all correlated to T ½ b[La] and no mutual relationship is evident (f*igure 4.6.1, r = 0.01)*. It was found to be very weakly correlated to counter movement jump height (r = 0.33).

T ½ b[La] shows a closer relationship to speed over 20m than it does to acceleration. However the correlation found in this study is too weak to be considered relevant or significant (*figure 4.6.2, r = 0.25).* However, speed displayed a close, positive correlation to counter movement jump height which was used to determine lower limb power (r = 0.69).

A modest negative correlation is evident with regard to T ½ b[La] and counter movement jump height (figure 4.6.3, r = -0.39*). Figure 4.6.3* shows a tendency for subjects with a longer T ½ b[La] to display a reduced counter movement jump height. As previously stated, counter movement jump height was positively well correlated to sprint performance at 20m (r = 0.69)

A weak negative correlation is observed between lower limb strength and the half-time of blood lactate removal (r = -0.39). This trend is clear in *figure 4.6.4* with individuals who are stronger relative to body weight showing a capacity to remove lactate quicker than their relatively weaker peers.

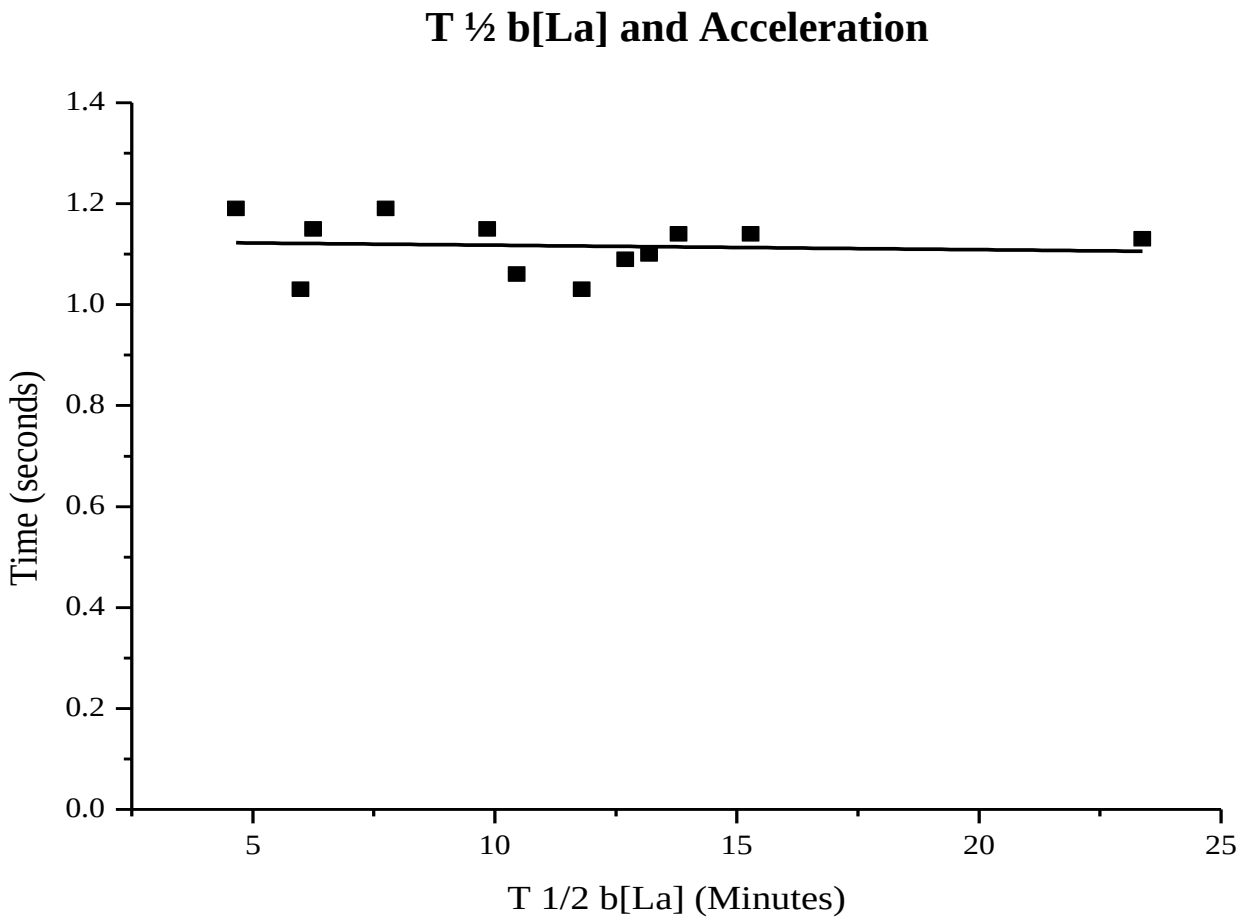

Figure 4.6.1: Trend between the time taken to clear blood lactate and 5m sprint time as a measure of acceleration.*5m v T ½ (r = 0.01)*

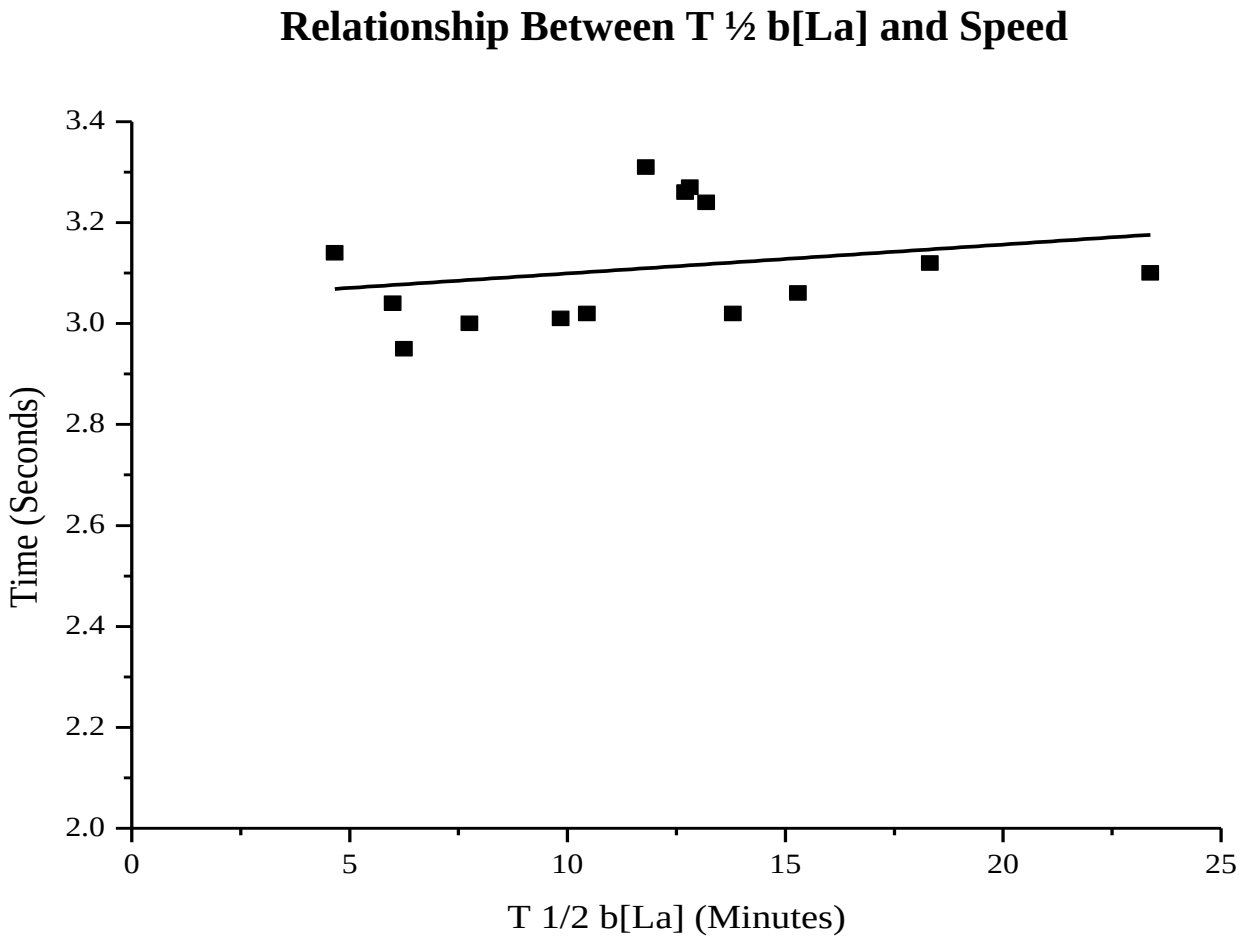

Figure 4.6.2: The trend between the time taken to clear blood lactate and speed as measured by 20m sprint performance
20m v T ½ (r = 0.25)

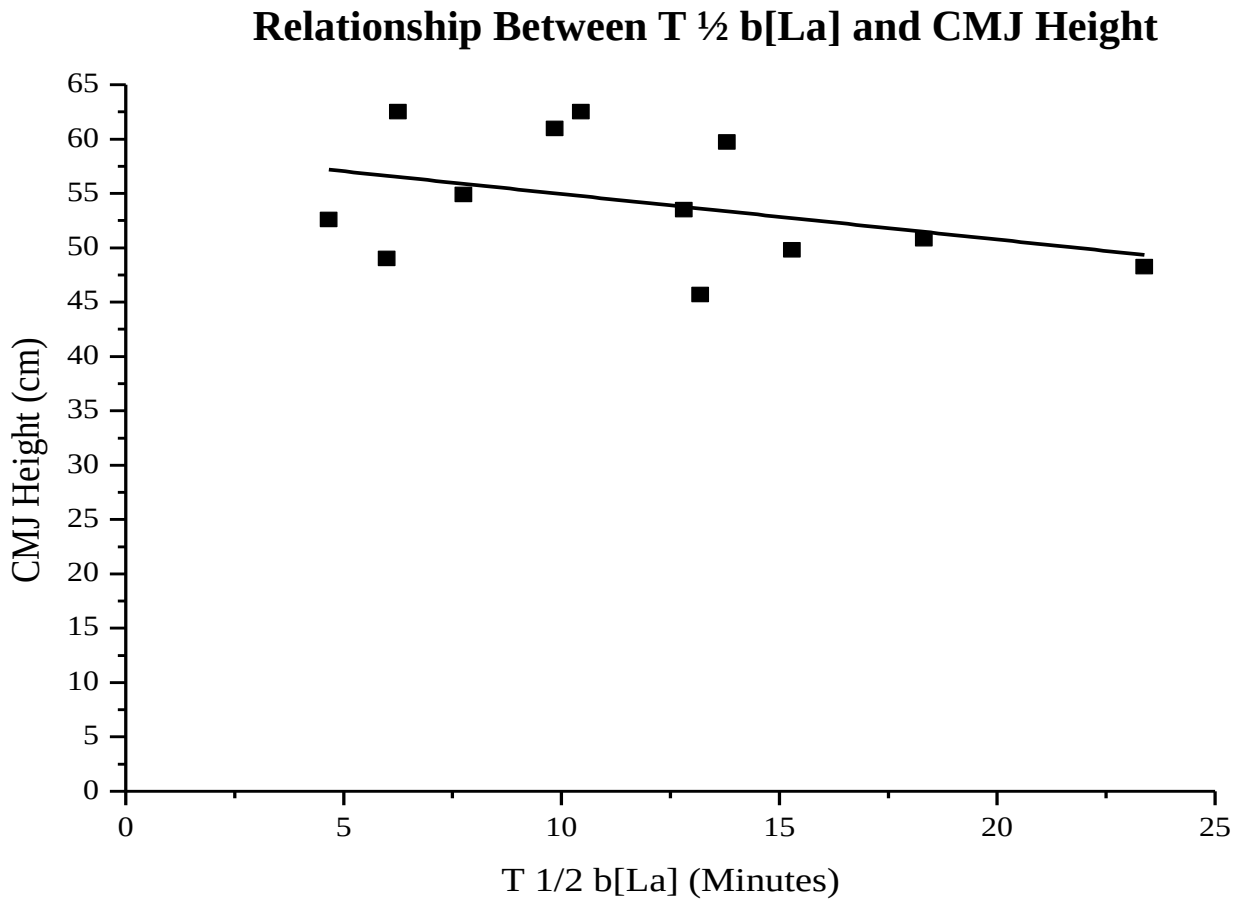

Figure 4.6.3: The nature of association between the half time of blood lactate clearance and limb power as measured by CMJ height *CMJ v T ½ (r = -0.39).*

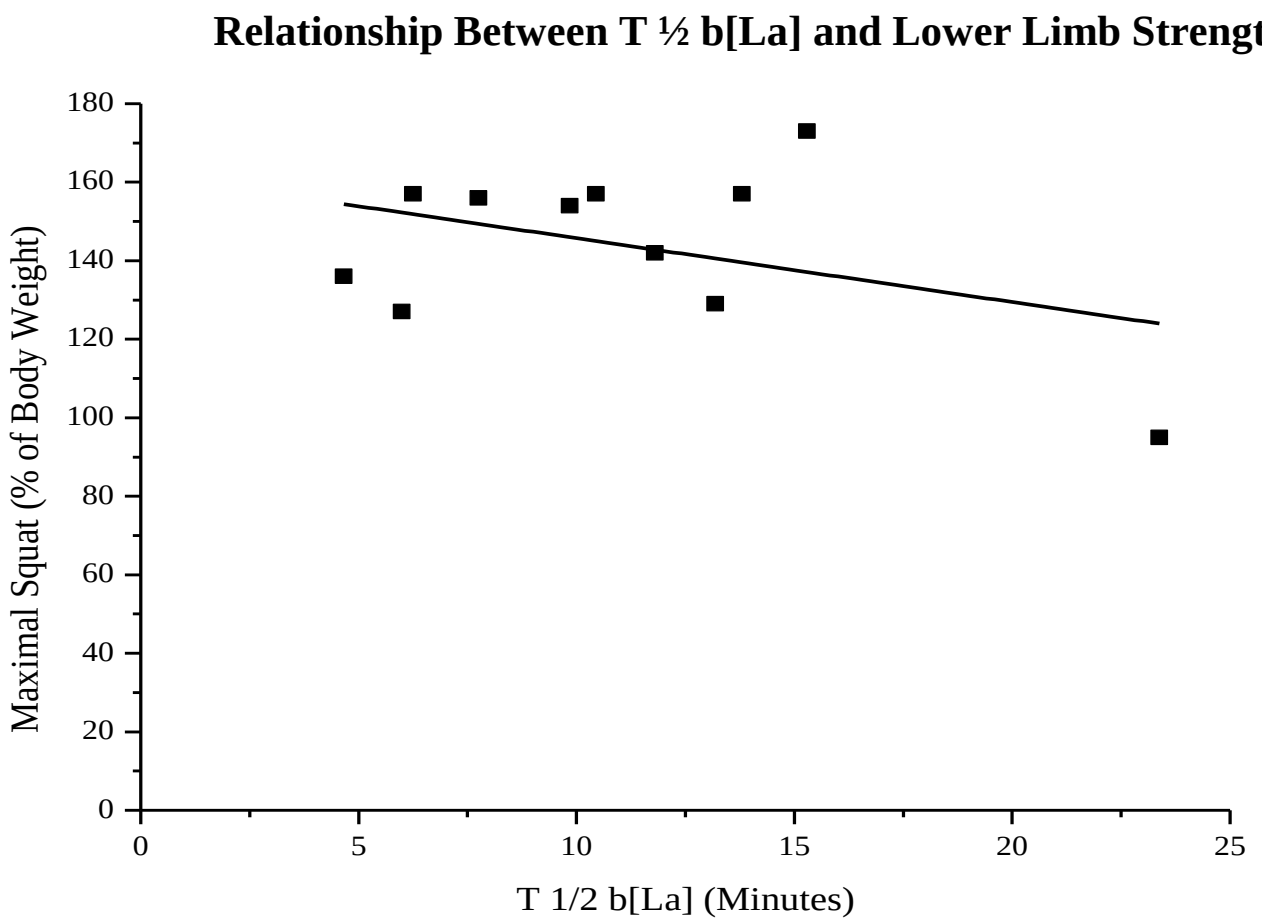

Figure 4.6.4: The trend between the half time of blood lactate clearance and lower limb strength as measured by maximal squat, expressed as a percentage of body weight (r = -0.39).

Chapter 5 - Discussion

5.1 Main Findings

This study aimed to test the hypothesis that ventilation may be used as a non-invasive measure of lactate clearance. The results returned suggest that, while neither variable is exclusively dependent on the other, they are intimately related to the extent that peak ventilation is indicative of the ability of an individual to remove lactate.

In addition, and equally as significant, it was found that the removal of lactate did not reflect the accepted "two compartment model" (Freund & Gendry, 1978) with only one distinguishable time course and therefore one process evident. This observation was confirmed by the fit of a number of the individual post exercise b[La] curves.

The resting values for the variables of interest shown in *table 4.1* indicate the physiological state of subjects prior to commencement of the protocol. Mean resting VO_2 and VCO_2 values are within the expected range and this, along with the normal resting minute ventilation, provides verification of the subjects comfort in the setting. Subjects were requested to eat and drink as normal in the hours preceding the test in an attempt to recreate standard pre-competition conditions. A resting RER of 0.77 and mean resting b[glu] of 4.51 $mmol.l^{-1}$ are reflective of the mixed substrate utilization and stable glucose metabolism typical of these athletes.

Resting b[La] is slightly higher than standard values. However, the large standard deviation from the mean confirms that this is likely to be indicative of the variation of the cohort as opposed to an inherent issue with the sample. It is highly unlikely that this is an artefact of "overtraining syndrome" as the lactate response is typically blunted in such athletes, giving a lower submaximal and peak b[la]. It has been suggested that this is a result of reduced muscle glycogen (Hackney et al, 1990; Costill, 1988). The $b[La]_{peak}$ and resting RER shown in *tables 4.1 and 4.2*, respectively, suggests this does not

apply to the cohort of this study. Investigation by Halson et al (2002) found that inducing a state of "over reaching" in endurance cyclists did not significantly alter resting b[La] and displayed similar values to the present study. This illustrates how the regularity of competition and training presents obstacles to maintaining a balance between prescribing intensive training programs and allowing for appropriate rest periods. The testing conditions replicated those likely to exist in a professional environment where it is not unexpected for resting b[La] to be elevated as it is impractical to implement significant rest periods during the testing bout, potentially leading to an element of residual fatigue. As will be discussed, however, the relationship between ventilation and T ½ b[La] is found to be strong in spite of this caveat. The ventilatory and b[La] measures at peak exercise, shown in *table 4.2,* validate the test as a peak test and are consistent with the literature, confirming the validity of the $b[La]_{peak}$ measurements. It should be noted that not all criteria were satisfied to consider test maximal (Howley et al, 1995).

Mean T ½ b[La] was 11.87 (±5.7) minutes. This represents a slower rate of clearance of blood lactate than is shown in previous studies by Gharbi et al (2008) and Belcastro and Bonen (1975) amongst others. However, the active recovery periods within these studies are not intensity matched and therefore this disparity is expected. Indeed, a study by Menzies et al (2010) found that the maximum rate of lactate clearance was close to the lactate threshold, substantially more intense (though possibly not as sport-specific) than the protocol in this study. It is anticipated that an active recovery period of higher intensity would reduce T ½ b[La] although the magnitude of the reduction is hard to ascertain. This is also dependent on being sure of the readiness of the players for assessment and does not take into account any residual fatigue from previous training and competition. In this study there was no requirement to maximise T ½ b[La], as the intention was to analyse its' natural dynamics. Therefore, the recovery period, like the exercise period itself, did not reflect match play.

Mean $b[La]_{peak}$ reached levels which are consistent with those attained by professional footballers during competition (Ekblom, 1986; Mclaren, 1987) therefore validating the specificity of the protocol used. After 15 minutes of active recovery, the mean b[La] was still significantly elevated from resting levels. Thus, the initial mechanisms employed to remove lactate from the working muscle in this sample are inadequate to re-establish resting metabolic state.

This is substantiated by the observation that 8 of the 14 subjects tested displayed a marked upward inflection in the post-exercise b[La] curve between the 3 and 5 minutes points during active recovery. From what has been established regarding the training status of the cohort, it is valid to infer that this is a consequence of an inability to use lactate as an oxidative substrate in the working muscle, leaving the prolonged hepatic clearance as the dominant mechanism. Taken in isolation, this may seem like a quirk of protocol or a measurement anomaly. However, assessed in conjunction with the kinetics of lactate clearance modelled using the biexponential function in *Eq 1*, it is clear that they are a result of defined physiological processes.

The lone time course shown conflicts with the "two compartment model" proposed by Freund and Gendry (1978) and discussed in detail by Oyono-Enguelle et al (1993) amongst others. This is strong evidence to suggest that only one process of lactate clearance/removal is operating efficiently in the recovery period. Using a similar recovery protocol to Menzies et al (2010) may provide more defined processes but was not practical in this study due to the fact that subjects were requested to run until volitional exhaustion. Given what has been established with regard to the training status of the sample and their characterization as athletes, it is proposed that the process which allows lactate to be oxidized by intracellular and cell-cell "shuttling" is inefficient. This is likely to be the result of a muscle fibre profile which does not possess the physiological properties conducive to the exchange of lactate (γ_1). The profile shown in *figure 3.1* is indicative of the two components shown in more specifically trained athletes and confirms the suitability of the protocol and apparatus used.

Measurement of VO_{2peak} may be indicative of central aerobic capacity and improved by widely accepted methods (McMillan et al, 2005) but does not reflect the peripheral aerobic capabilities of the athlete as evidenced by the relationship in *figure 4.4.3*. While oxidation of lactate can account for around up to75% of its clearance during exercise (Brooks 2009), this is not possible without a sufficient pool of MCTs to transport it to oxidative sites and LDH to catalyse the reaction. Subjects are therefore overly reliant on the slow phase (γ_2) which is insufficient to maintain the decline in b[La] seen in *figure 3.1*. Even this process was shown to be upregulated in individuals with a higher muscle oxidative capacity by Thomas et al (2004). Evidently, the cohort is one which has adapted to the demands placed on them by the sport. They have respectable aerobic capacity, are strong, fast and powerful yet show unexpected lactate clearance profiles. The significance of this observation will be discussed in depth in this discussion.

It has been reported that the complexity of the relationship between ventilation and lactate kinetics determines that there is great difficulty in apportioning causality to one particular variable or process (Gaesser & Brooks, 1984; Meyer, 2004). The results provided do not disprove this assessment and should not be interpreted as suggesting the rate of lactate clearance is determined by ventilation. Rather, peak ventilation can be used as a convenient, indirect measure of lactate clearance in this population. The swathe of historical studies which resolved that this was a result of lactate causing an increase in [H+] and therefore reducing cellular pH (Davis, 1985) have been remodelled to remove culpability for this phenomenon from lactate (Rogbergs, 2004). With pyruvate acting as an acceptor of two H+ ions as it is reduced to lactate, it is clear that lactate formation by this process attenuates acidosis. Indeed, it has been shown that the ability to oxidize pyruvate is strongly correlated to lactate threshold (Ivy & Costill et al, 1980). However, regardless of its genesis, the rise in [H+] concurrent with lactate accumulation must be buffered and herein lays the explanation for ventilation predicting the rate of lactate clearance. The excess CO_2 produced in the bicarbonate buffering reaction is an added stimulus to the central chemoreceptors driving ventilation. Evidently this process will be taxed more strenuously in individuals who display an inability to clear lactate (*figure 4.4.1)*. This is in

agreement with the work of Casaburi and Wasserman (1987) who cautiously established a link between ventilation and lactate while acknowledging the significance of other variables. While this research appropriately describes the use of ventilatory measures with regard to lactate accumulation during exercise, the findings shown previously translate this to make inferences on the rate of lactate clearance.

The use of lactate as a fuel source during high intensity exercise is an established and accepted concept (Brooks, 1988; Brooks & Gaesser, 1980; Gertz, 1988). Superficially it seems that the metabolism of this supplementary substrate could stimulate ventilation through its by-products. However, RER_{peak} is not well correlated with Ve_{peak} or T ½ b[La], likely to be as a result of the rapidly diminishing contribution of substrate metabolism to ventilation at supramaximal levels where peripheral receptors provide ventilatory feedback (Wasserman, 1987). The lack of information provided by RER_{peak} at supramaximal levels is at least partially indicative of the altered metabolic environment. The multi-factorial nature of gas exchange at these intensities is such that using peak minute ventilation as an indicator of cellular processes provides a larger room for error.

The extensive body of work produced by Brooks et al (1986; 1989; 1998; 1999; 2009) has done much to elucidate the mechanisms of lactate clearance in extreme detail. The efficient clearance of lactate during exercise is highly reliant upon its shuttling to oxidative fibres by monocarboxylate transporters (MCTs). It has been verified on a number of occasions that MCT activity and concentration is enhanced by endurance training (Dubouchaud, 2000; Bonen, 2001; Pilegaard, 1999). The increased mitochondrial density engendered by this training is likely to indirectly give a relative increase in [LDH]. Consequently, it has been asserted that endurance trained athletes with a high VO_{2peak} show more efficient lactate clearance. This is not corroborated by the results of this investigation as the converse trend is observed (*figure 4.4.3).* It is contended that this is indicative of a tolerance of increased b[La] which allowed for a longer time to exhaustion (*figure 4.4.4)* as opposed to alterations

(or lack of) at a cellular level. Despite their status as professional football players, the cohort did not undertake training which could be considered adequate to provoke a significant peripheral aerobic adaptation. Much of the aerobic conditioning followed the philosophy of Hoff et al (2004) with 4x4 minute bouts of intense exercise. This method of training focuses predominantly on central adaptations to increase aerobic capacity. As such, the enzymatic profile of subjects is unlikely to display the concentration of LDH and MCT which are desirable for a rapid T ½ b[La] despite highly respectable VO_{2peak} values which are determined by the central factors such as cardiac output. This seems to reinforce the importance of rate of b[La] clearance compared to VO_{2peak} with regard to athletic performance in football.

The issue of tolerance of lactate is one that should be extensively examined. The results provided in this study suggest that the training programs these athletes are subject to are possibly likely to engender an ability to tolerate lactate as opposed to an ability to remove it in the manner observed in highly trained athletes. The regularity and short duration of the high intensity efforts may make it as beneficial to tolerate increasing b[La] as remove it. This supposition needs further investigation before inferences can be made with great certainty. It is, however, supported by sound theory and the results displayed previously.

The dynamics of the training undertaken by the cohort are sufficient to improve these central determinants without the underlying peripheral adaptations at a cellular level. Any aerobic training implemented followed the principles of McMillan et al (2005) who displayed that these adaptations can be accrued in a period of around 10 weeks and so it is considered that the 6 month training period prior to testing was more than suitable to allow the cohort to be representative of the wider population. This also provides an explanation for the modest mean lactate threshold found in the sample as the sample are not as metabolically efficient as their aerobically trained counterparts.

Mean lactate threshold occurred at 62.5% (±8.7%). This illustrates well the respectable yet limited physical capability of the cohort. This figure would be markedly higher in an endurance trained population with a greater economy of movement. However, this group are not trained to run at gradually increasing speeds, in straight lines or on a gradient and this measure is therefore not entirely applicable to the specific demands of the sport. Also, when lactate threshold occurs at this point, it is not related to the ability to clear lactate. This seems to suggest that the point at which the ventilatory response to lactate accumulation occurs is irrelevant to rate at which it is subsequently cleared. This is not entirely expected as the accumulation of lactate is symptomatic of an inability to oxidize lactate in the muscle during exercise by the process also used for lactate exchange in the post exercise period. The obvious implication here is that this process is highly under-employed by the cohort, causing a divergence in the relationship between T ½ b[La] and the lactate threshold and substantiating the previous assertion of the single time course for lactate clearance.

It is clear that this sample cannot be considered aerobically well trained and is unlikely to display muscle histology akin to endurance athletes, despite some subjects attaining more than adequate MAS times. The training history of the population dictates that strength training took precedence over aerobic conditioning. The emphasis on strength training was compounded by progressive, individualised, periodised strength training programs for all athletes. The product of this philosophy is apparent with only one subject failing to squat at least 100% of body weight. The group tested can be depicted as athletes with elements of both aerobic and anaerobic qualities which are developed to varying degrees across the cohort. While in general terms the cohort is located somewhat centrally on a scale of aerobically and anaerobically trained athletes, there is a natural interpersonal inequality.

The lack of distinct relationship between acceleration and the removal of blood lactate (*figure 4.6.1)* is likely to be indicative of the size and training history of the sample. This can also be applied to the inability to detect a significant relationship between T ½ b[La] and speed over 20m. The high

proportion of glycolytic, type II fibres that is required for optimal acceleration and sprint performance is comparable to the profile necessary to achieve a superior counter movement jump height, as evidenced by the strong correlation between CMJ and 20m sprint performance (r = 0.63).

The fact that lower limb power (CMJ height) and lower limb strength (squat) show the beginnings of a negative correlation with T ½ b[La] (r = -0.39 & r = -0.39) suggests that this may be more indicative of the muscle fibre characteristics of the cohort. This trend and the positive association T ½ b[La] shows with MAS time (r = 0.48) and VO_{2peak} (r = 0.48) is at odds with studies which suggest endurance trained athletes with high oxidative capacity clear lactate more rapidly than power trained athletes (Taoutaou et al, 1995; Brooks, 1998, 1999). However, such research is generally carried out on experienced athletes who have undergone intensive and specific aerobic or anaerobic training and are therefore not directly comparable to the results presented here. This auxiliary data is used as a proxy, indirect measure of the physical qualities of each subject and is presented against lactate clearance to display any potential relationships between specific physiological capabilities and the ability to remove lactate. Again, these findings highlight the previous assertion that the cohort in this study can be described as "generally trained". This is reflective of the multi-faceted demands of the sport where a cross-section of physical qualities is advantageous. For the majority of players, an aerobic capacity comparable to that of an endurance athlete and to a lesser extent the speed of a power trained athlete is of little practical benefit if it is not accompanied by proficiency in the range of other technical, physical and psychological qualities.

Limitations

The majority of investigations into lactate kinetics are, to a certain degree, compromised by the use of blood lactate as a measure of activity in the muscle. For this purpose and for accurate characterisation of the muscle fibre type of athletes in the sample, the use of muscle biopsies would have proved beneficial. However, the protocol and apparatus used were found to be more than sufficient for the

purposes of this study. The sample size was dictated by the size and physical wellbeing of the squad of players. A larger sample of physically mature athletes may make the findings more applicable to elite level sport.

Conclusions

In team sports, or sports which do not provide an environment appropriate for blood sampling, peak ventilation can provide an indirect estimate of lactate clearance ability. This permits lactate clearance to be estimated concurrently with maximal oxygen uptake, lactate threshold and a range of other ventilatory parameters. Furthermore, the nature of the training required for physically diverse intermittent sports seems insufficient to impart the enzymatic profile necessary to maximise lactate clearance.

Directions for Future Research

The findings of this study provide a foundation for further, in depth research. It may be possible to quantify the rate of lactate clearance for a given peak ventilation and therefore provide a more detailed description the physical capabilities of an individual athletes.

An intervention study can be developed which will allow athletes of intermittent sports to undergo aerobic training determined as being sufficient for cellular adaptation within the boundaries of standard pre-season and on-season training. Such an investigation will allow dissemination of whether intermittent and endurance training are necessarily mutually exclusive.

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Appendix A

College of Medical, Veterinary & Life Sciences Ethics Committee for Non Clinical Research Involving Human Subjects

APPLICATION FORM FOR ETHICAL APPROVAL

Project Title: *"Can Ventilation be Used as a Non-invasive Measure of Lactate Clearance".*

Is this project from a commercial source, or funded by a research grant of any kind? Yes/No

If yes,

a) Has it been referred to Research & Enterprise?

Has it been allocated a project Number?

b) Give details and ensure that this is stated on the Informed Consent form.

Insurance Restrictions.

The University insurance cover is restricted in certain, specific circumstances, e.g. the use of hazardous materials, work overseas and numbers of participants in excess of 5000. All such projects must be referred to Research and Enterprise before ethical approval is sought.

Date of submission

12/9/11

Name of all person(s) submitting research proposal

Mark Gallagher

Dr. Niall MacFarlane

Position(s) held

Mark Gallagher – Student

Dr. Niall MacFarlane – Senior University teacher

Department/Group/Institute/Centre

University of Glasgow/School of Life Science/College of Veterinary and Life Sciences

Address for correspondence relating to this submission
Dr Niall G MacFarlane
Room 240A, West Medical Building,
College of Medical, Veterinary and Life Sciences,
Glasgow University,

Glasgow

G12 8QQ

Email address: 0700085g@student.gla.ac.uk

Name of Principal Researcher (if different from above e.g., Student's Supervisor)

Niall MacFarlane

Position held

Senior University teacher

Undergraduate student project **Yes/<u>No</u>**

Postgraduate student project **<u>Yes</u>/No** **MSc**

1. **Describe the purposes of the research proposed. Please include the background and scientific justification for the research. Why is this an area of importance?**

Blood lactate concentration ([La]) increases to around 7-8 mmol.l^{-1} during bouts of intense, intermittent, exercise common in football games and training (Ekblom et al, 1986). This results from an imbalance between lactate production and clearance. This increase in [La] is associated with the onset of fatigue and reduced performance and is often used as a performance marker in athletic populations and to set relative training intensities. Lactate clearance is achieved in working muscle by conversion to pyruvate via lactate dehydrogenase or through gluconeogenesis in the liver. Lactate clearance is normally measured by time consuming and invasive (albeit minimally) methods that directly measure [La] over time (reducing the applicability of such measurements in team sport settings).

Ineffective lactate clearance can lead to lactic acid accumulation and dissociation of the acid into lactate and H^+ ions. Sodium bicarbonate buffers these H^+ ions produced in a reaction that produces H_2O and CO_2. The CO_2 is expelled in a process called respiratory compensation that stimulate the central chemoreceptors to augment ventilation (Whipp and Ward, 1991). This observation may allow an easier, less invasive, method of assessing lactate clearance by measuring peak ventilation and respiratory exchange ratio during testing of maximal oxygen uptake. So the aim of this study is to determine whether a relationship exists between ventilation, respiratory exchange ratio and lactate clearance. A strong relationship will allow us to use these simple, non-invasive, markers to predict lactate clearance in athletic populations and subsequently apply and test training interventions that improve lactate clearance where necessary.

Lactate clearance is highly significant in high intensity, intermittent exercise where recovery from preceding bouts of activity will influence performance in subsequent bouts. Strategies to optimise lactate clearance have been proposed in animal (Donovan and Brooks, 1983 and human studies (Menzies and Kemi et al, 2010). However, testing the effects of such interventions has been a limiting factor in some athletic populations. Removing this barrier would provide data to support athlete development.

References

Donovan and Brooks. Endurance training affects lactate clearance, not lactate production. American Journal of Physiology, 1983, 244 (1), E83-E92.
Ekblom et al. Applied Physiology of Soccer. Sports Medicine, 1986, 3(1), 50-60.
Menzies and Kemi. Blood lactate clearance during active recovery after an intense running bout depends on the intensity of the active recovery. Journal of Sports Sciences, 2010, 28 (9), 975-982.
Whip and Ward. Determinants and Control of Breathing During Muscular Exercise. British Journal of Sports Medicine, 1998, 32, 199-211.

2. Describe the design of the study and methods to be used. Include sample size and the calculation used to determine this. Statistical advice should be obtained if in doubt.

Subjects

Subjects will be recruited from the Professional Youth Academy of Celtic Football Club. These players will all be members of the Under 19 and Under 17 squads and therefore signed on professional contracts. Under the terms of their employment, all subjects will have completed the medical screening process of the Scottish Football Association including echo, ECG and blood tests.

Protocol

Subjects will be required to attend for a single testing period. Subjects will complete a treadmill ramp test which will be terminated at the point of volitional exhaustion during which expired gas and ventilation will be analysed with continuous heart rate and regular blood pressure monitoring. Ratings of perceived exertion will be taken at regular intervals. A period of active recovery set at 5 Kmh^{-1} on a 1% gradient will follow the maximal test until blood lactate returns to normal levels. During this period, capillary blood samples will be taken at peak, 1 minute, 3 minutes, 5 minutes and 15 minutes post exercise using a finger prick method. Blood samples will be analysed using a GM7 lactate analyser or similar to calculate lactate clearance.

Familiarisation

No period of familiarisation will be required as each player is used to such stress testing (they are expected to undertake such protocols at regular intervals under the terms of their employment).

Sample Size

A minimum of 13 players would be required for this study to show differences in ventilation, respiratory exchange ratio and lactate clearance after an intervention (based on the standard deviation of the independent variable and dependent variables from our previous data to obtain 90% power at 5% significance levels). However, no intervention is planned at this juncture and we aim to obtain a simple correlation co-efficient to estimate the strength of the relationship between lactate clearance and ventilation or respiratory exchange ratio and we will recruit as many players as possible from the U19 and U17 squads.

The strength of correlation from this data will allow provide pilot data for us to establish a rationale for modelling lactate clearance from non-invasive data.

3. Describe the research procedures as they affect the research subject and any other parties involved.

It should be clear exactly what will happen to the research participant, how many times and in what order.

Subjects will be requested to complete one incremental exercise test on a treadmill to the point of exhaustion and so players will feel significantly out of breath. This is within the boundaries of normal training for the subjects chosen who are accustomed to high physical stress and will recover quickly. Gas exchange apparatus will be used to measure ventilation requiring the use of a mouth piece throughout the test. At 5 points after cessation of exercise small blood samples will be taken via a thumb prick procedure. Due to this procedure, only one finger prick will be required as the clot can be disturbed to stimulate blood flow and to give numerous samples.

4. How will potential participants in the study be (i) identified, (ii) approached and (iii) recruited?

Give details for cases and controls separately if appropriate:

i) All participants are trained athletes with experience of exercise testing.

ii) Participants are members of the youth academy of Celtic Football Club and are approached through the club.

iii) Information sheets are distributed to each player in the squad detailing the procedure and giving them the option to participate.

5. What are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

Thumb prick blood samples may be slightly uncomfortable.

Although participants are used to the physical demands of the protocol and are regularly tested under the terms of their employment, this data is not usually intended for publication. Therefore there may be an element of feeling obliged to allow the use of data despite their right to opt out.

6. Outline the reasons why the possible benefits, to be gained from the project, justify any risks or discomforts involved.

The main benefit to be gained from the study will be the potential to measure blood lactate clearance through non-invasive measurement of ventilation. This

will provide a more practical protocol for future research into blood lactate concentration during exercise.

7. Who are the investigators (including assistants) who will conduct the research? What are their qualifications and experience?

Mark Gallagher - BSc Hons Physiology and Sports Science

Dr. Niall MacFarlane - BSc PhD – 20 years + experience of exercise physiology

8. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

First aid equipment will be available and the protocol will be within the Health and Safety Procedures of Celtic Football Club.

9. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital), describe how you intend to get this information. Include, where appropriate, which Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.

NA

10. Specify whether subjects will include students or others in a dependent relationship and where possible avoid recruiting students who might feel to be or be construed to be under an obligation to volunteer for a project. This is most likely to be where a student is enrolled on a course where the investigator is a teacher. In these circumstances the recruitment could be carried out by one of the other investigators or a suitably qualified third party.

All subjects are employed by Celtic Football Club and are contractually obliged to undertake such tests as part of their employment. These tests are within the parameters of the normal training schedule and as a result players are comfortable the requirements.

11. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects, and include documentation of the suitability of those researchers who will be in contact with children (eg Disclosure Scotland).

N/A – all the players are over 16 years of age. However, the staff involved have all been disclosure checked due to their activities in the Youth Academy with younger age groups.

12. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering an incentive.

Not for the study – but the athletes are all professional athletes carrying out the test as part of there normal training and testing activities

13. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

A subject information sheet will be provided with each participant providing a signature to confirm their willingness to allow the data obtained from these testing procedures to be used in this study.

14. Comment on any cultural, social or gender-based characteristics of the subject, which have affected the design of the project or may affect its conduct.

NA

15. Please state who will have access to the data and what measures will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g. will the data be anonymised, how will it be stored, how will access be restricted, and for how long will it be retained?

The data generated will be the property of Celtic Football Club and as such will be available to all medical, sports science and coaching staff. Any data used in a scientific report will be anonymous and each subject will have the option of opting out of the publication of their data

16. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

No

17. Proposed starting date

1/2/12

Expected completion date

1/9/11

18. Please state location(s) where the project will be carried out.

Celtic Park, Kerrydale Street, Glasgow

19. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g. where blood samples are being taken

All blood samples will be taken using sterilised equipment with all researchers wearing protective gloves. All procedures will follow the health and safety standards of Celtic Football Club.

20. Please state all relevant sources of funding or support for this study

Celtic Football Club

21 a). Are there any conflicts of interest related to this project for any member of the research team? This includes, but is not restricted to, financial or commercial interests in the findings. If so, please explain these in detail and justify the role of the research team. For each member of the research team please complete a declaration of conflicts of interest below.

Researcher:

Name: Mark Gallagher_____conflict of interest No

If yes, please detail below

Researcher:

Name: Niall MacFarlane_____conflict of interest No

If yes, please detail below

Researcher:

Name: _____conflict of interest Yes / No

If yes, please detail below

Researcher:

Name: _____conflict of interest Yes / No

If yes, please detail below

21 b). If there are any conflicts of interest, please describe these in detail and justify conducting the proposed study.

NA

22. How do you intend to disseminate the findings of this research?

All findings will be presented in the form of a scientific paper.

I confirm that have read the University of Glasgow's Data Protection Policy [http://www.gla.ac.uk/services/dpfoioffice/policiesandprocedures/dpa-policy/]

Please initial box

NM

Signed  Date 20/1/11

(Proposer of research)

For student projects

I confirm that I have read and contributed to this submission and believe that the methods proposed and ethical issues discussed are appropriate.

I confirm that the student will have the time and resources to complete this project.

Signed Date 20/1/11

(Supervisor of student)

Appendix B

UNIVERSITY of GLASGOW

College of Medical, Veterinary and Life Sciences

School of Life Sciences

Subject Information Sheet

Does Ventilation Predict Lactate Clearance?

This means, is it possible to find out how quickly lactate, which builds up and causes discomfort in the working muscle, is removed by measuring the rate of breathing?

You are invited to take part in the study outlined above. Make sure that you understand the information and if anything is not made clear then do not hesitate to ask. Please read the information provided within this document and consider whether you wish to participate. Thank you for taking the time to read this.

During intense exercise, there is a build up of a substance called lactate in the muscle. This causes discomfort and stops the muscle working to its peak. You will be familiar with this from previous training. The speed at which it is removed from the muscle is usually determined by taking blood samples. However, one method of removal is by increasing the

rate of breathing to breathe out carbon dioxide. Measuring this may be far easier in a football setting rather than taking blood samples. Therefore, we intend to study whether ventilation i.e. the rate of breathing, can tell us how quickly lactate is being cleared from the working muscle. This will vary from person to person for a number of different reasons but will allow us to gain a physiological profile for each player which you can use as you wish.

You have been invited to take part in this study due to your status as a well trained athlete and employee of Celtic Football Club. At least 13 other participants have also been chosen for the same reason.

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.
The study requires you to undergo one test. The length of this test will vary dependent on each subject. You will be placed on a treadmill and asked to run at gradually increasing speeds until the point where you feel you cannot run anymore. At regular intervals you will be asked to rate how hard you find the exercise on a scale of 1-20. We will measure your heart rate and blood pressure during the exercise. Following the test, a number of small blood samples will be taken via a thumb prick method. The only thing asked of the participant is to make sure they have run for the maximum length of time.

Following the test, you should make sure that you rehydrate to replace any lost fluids. No dietary restrictions will be imposed and normal daily activities can be resumed as soon as you feel adequately recovered. If there is a reason you do not think you are suitable for any of the aforementioned tasks then please make it known.

There are few risks to be considered when taking part in the study because as a trained athlete, you will be under no abnormal stress. The thumb prick to sample blood may cause very minimal discomfort and may leave a small mark on the skin.

There will be no physiological benefit gained from taking part in the study. However, the results obtained may be useful in developing further training programs.

All information, which is collected, about you during the course of the research will be kept strictly confidential and will only be available to club officials. Any information about you will have your name and address removed so that you cannot be recognised from it and you may refuse permission for the data produced to be published.

The results obtained may be published in scientific literature depending on the findings and may lead to further research on the topic. The full report will be available upon request

from any of the investigators. In the event of the study being published, you will still not be identified.

The research is being sponsored and funded by Celtic Football Club Ltd working in partnership with the University of Glasgow.

The study has been reviewed and approved by the ethics committee for the College of Medical, Veterinary and Life Sciences.

Thank you for taking part in the study.

Contact:
Mark Gallagher
e-mail: 0700085g@student.gla.ac.uk

Appendix C

UNIVERSITY of GLASGOW

College of Medical, Veterinary and Life Sciences

School of Life Sciences

Centre Identification Number:
Study Number:
Subject Identification number:

Consent Form

Title: Does Ventilation Predict Lactate Clearance?
Researchers: Mark Gallagher
Dr. Niall MacFarlane

Please Initial Box

1. I confirm that I have read and understand the information sheet dated ..... for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my legal rights being affected. ☐

3. I agree to take part in the above study. ☐

Name of Subject	**Signature**	**Date**
Name of Person Taking Consent	**Signature**	**Date**
Researcher	**Signature**	**Date**

Name:
Date of Birth:
Height:
Weight:

b[La] $mmoll^{-1}$	
Resting	
Peak	
1 min post ex	
3 min post ex	
5 min post ex	
15 min post ex	

RER	
Resting	
Peak	
1 min post ex	
3 min post ex	
5 min post ex	
15 min post ex	

Ve (l/min)	
Resting	
Peak	
1 min post ex	
3 min post ex	
5 min post ex	
15 min post ex	

VO2 (m/Kgl/min)	
Resting	
Peak	
1 min post ex	
3 min post ex	
5 min post ex	
15 min post ex	

VCO2 (ml/Kg/min)	
Resting	
Peak	
1 min post ex	
3 min post ex	
5 min post ex	
15 min post ex	

b[Glu]	
Resting	
Peak	
1 min post ex	
3 min post ex	
5 min post ex	
15 min post ex	

RPE	
2 min	
4 min	
6 min	
8 min	
10 min	
12 min	

HR	
2 min	
4 min	
6 min	
8 min	
10 min	
12 min	

Appendix E

Rate of Perceived exertion (RPE)

Rating	Perception
6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Appendix F

Table 4.2: Characteristics of Each Individual Subject Obtained During Testing Protocol.

	$T^{1/2}$ [La]	RER_{peak}	Ve_{peak} (l/min)	$[La]_{peak}$ (mmoll-1)	VO_{2peak} (ml/Kg/min)	LT (%VO2max)	b[Glu] (mmoll-1
Subject 1	**15.29**	**1.79**	**190**	**10.2**	**63.65**	**78**	**3.6**
Subject 2	**9.85**	**1.39**	**170.1**	**13.8**	**64**	**72**	**5.3**
Subject 3	**4.66**	**1.29**	**130.1**	**5.1**	**64**	**82**	**4.1**
Subject 4	**13.8**	**1.29**	**152.6**	**9.5**	**71.54**	**63**	**4.7**
Subject 5	**6.25**	**1.66**	**140.6**	**6.6**	**52.5**	**58**	**4.7**
Subject 6	**7.75**	**1.55**	**135.1**	**10.7**	**54.67**	**63**	**5.8**
Subject 7	**10.45**	**1.55**	**150.3**	**7.4**	**41.1**	**78**	**5.3**
Subject 8	**5.99**	**1.52**	**112.3**	**6.2**	**52.58**	**50**	**3.4**
Subject 9	**11.8**	**1.21**	**130.7**	**9.9**	**61**	**63.2**	**3.6**
Subject 10	**13.19**	**1.22**	**134.2**	**13.2**	**64**	**66**	**5.7**
Subject 11	**12.7**	**1.13**	**125**	**18**	**65**	**58.8**	**3.8**
Subject 12	**12.81**	**1.25**	**169.7**	**6.5**	**66**	**64.1**	**4.3**
Subject 13	**18.32**	**1.25**	**152.6**	**6.9**	**66**	**65.4**	**4**
Subject 14	**23.38**	**1.42**	**187.9**	**8**	**82.5**	**67**	**4.9**

Table 4.2: Pearson's correlation coefficient to determine the strength of the relationship between physiological variables on interest.

	$T^{1/2}$ [La]	RER_{peak}	Ve_{peak}	$[La]_{peak}$	VO_{2peak}	LT	$b[Glu]_{rest}$
$T^{1/2}$ [La]	-	-0.2	0.7	-0.3	0.5	-0.1	-0.1
RER_{peak}	-0.2	-	0.3	-0.3	0.5	-0.1	-0.1
Ve_{peak}	0.7	0.3	-	-0.1	0.2	0.0	0.2
$[La]_{peak}$	-0.3	-0.3	-0.1	-	0.2	0.0	0.2
VO_{2peak}	0.5	0.5	0.2	0.2	-	0.0	0.2
LT	-0.1	-0.1	0.0	0.0	0.0	-	0.2
$b[Glu]_{rest}$	-0.1	-0.1	0.2	0.2	0.2	0.2	-

$T^{1/2}$[La] = Half-time of blood lactate clearance; LT = Lactate Threshold; Ve_{peak} = Peak minute ventilation attained

Appendix G

The power of the calculation was calculated as follows:

Significance level: 0.05 (two sided)

Standard deviation of the dependent variable: 18

Standard deviation of the independent variable: 1.08

Power: 0.9

Minimal detectable difference: 17.3

That is, the probability is 90 percent that the study will detect a relationship between the independent and the dependent variables at a two-sided 0.05 significance level, if the true change in the dependent variables is 17.3 units per unit change in the independent variable.