LACTATE ACCUMULATION DURING EXERCISE - THE INFLUENCE OF BODY FLUID SHIFTS.

Barbara Ann Castleman

A Research Report submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg in partial fulfilment of the degree of M.Sc. (Med).

October 1998

DECLARATION

I declare that this research report is my own, unaided work except to the extent indicated in the acknowledgements. It is being submitted in partial fulfilment for the degree of Master of Science in Exercise Physiology at the University of the Witwatersrand, Johannesburg. It has not been submitted previously as a requirement for any degree or examination in this or any other university.

.....25th.....day of .....June....., 19.99...

.....

(Barbara Ann Castleman)

I certify that the study contained in this research report has the approval of the Committee for Research on Human Subjects, Clearance certificate number M931007 (See Appendix)

..... Date: 25/6/99

(Signature of student)

..... Date: 25 June 99.

(Signature of supervisor)

ABSTRACT

During graded exercise, an intensity is reached where a subjects ability to remove lactate lags behind the rate of lactate production. The influence of body fluid shifts, during exercise of increasing intensity, on the pattern of the blood lactate response was studied.

The maximal oxygen uptake (VO_2 max) was measured using a treadmill, on eleven subjects. Subsequently, lactate accumulation in venous blood was measured, in triplicate, up to an oxygen consumption greater than 90% VO_2max. During all exercise, oxygen consumption was measured using an online system. In addition, the blood samples at each workload were used to determine haematocrit (Hct) and haemoglobin (Hb) levels.

The Hct and Hb values were used to calculate lactate accumulation (corrected for body fluid shifts) as opposed to the absolute or total lactate levels. The correction for body fluid shifts was done using two techniques. The one using haematocrit only and the other using both haematocrit and haemoglobin. The total and accumulated lactate levels were related to %VO_2max using two different models. Firstly, a lactate threshold (LT) was determined using the classic lactate turning point (LTP) concept, (ie. two straight lines fitted to the data points). These lines

were computer generated. The intercept of the two lines (LT) was compared for total lactate against accumulated lactate (calculated using Hct alone and secondly Hct in combination with Hb. In the latter cases, both the LT intercepts were shifted slightly to the right (ie. to a higher % of VO_2max). The average difference in LT when adjusting with Hb and Hct was 0,519 of %VO_2max (0,72% change) and when adjusting with Hct only was 1,17 of %VO_2 max (1,65% change).

Secondly, an exponential curve was fitted by regression to the data (r=0.989+/-0.018). A substantial shift in the curve, both down and to the right, was obtained when adjusting total lactate to accumulated lactate. The %VO_2 max at a lactate concentration of 4mmol/l was used to define the position of the curve. The difference when using Hct alone to calculate accumulated lactate corrected for fluid shift was - 9,20% of VO_2max ($p<0.05$), and when using Hb and Hct in combination, -8,71% of VO_2max ($p<0.05$).

It is concluded that expressing the lactate curve as an accumulated curve (corrected for body fluid shifts), rather than in absolute terms, significantly alters the construction of the curve during the exercise protocol used in this study. This is especially relevant when using the exponential model.

ACKNOWLEDGEMENTS

I gratefully acknowledge the assistance of the following people:

- My supervisor, Professor G. Rogers, who has provided invaluable guidance and support.

- Sr. Fikhile Makhaye and Dr. Natasha Thomas for their help during the exercise testing in the laboratory.

- The athletes who volunteered their free time to participate in this study.

- The Medical Faculty Research Endowment Fund for funding.

- My family, Alan, Melissa and Matthew, for their support, encouragement and understanding throughout my studies.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1. INTRODUCTION

Many factors have been identified as having an influence on success in distance running. These include VO_2 max, fraction of VO_2 max that can be utilised and running economy, which have all been associated with successful performance (1,7,12,19). It has been recognised since 1927 that lactate increases in the blood once the exercise intensity rises above a certain level. This accumulation of lactate has been used as a gauge of physical fitness in patients with cardio-vascular disease as well as in normal, healthy subjects to assess the effects of training (1,5,11,12,15,30,59). The ability to perform at a higher percentage of VO_2max without the accumulation of lactate in the muscle and blood, has a high correlation with success in distance running (1,22,28,53,61,79,80).

Lactate production occurs as a result of the rapid breakdown of glycogen in the presence or absence of oxygen (14,18,23,33,38,51). The intensity of the exercise that begins to elicit the increase in lactate concentration in muscle and blood is highly variable, and is influenced by many factors, including diet, physical fitness and the type and duration of the exercise (1,2,6,16,31,44,52,52,78).

There has been considerable debate concerning the pattern of lactate accumulation, with two basic schools of thought emerging

(i) the anaerobic threshold (classical theory) which proposes that at a certain work intensity, aerobic metabolic processes can no longer meet the skeletal muscle requirements for ATP, and anaerobic glycolysis, with the accompanying lactate production, must supplement aerobic metabolism (3,4,88,89), and (ii) the imbalance between production and removal of lactate which results in an exponential increase in lactate (8,9,10,13,25,27,28,46,60).

It has been known for some time that during short, intense exercise, fluid is lost from the vascular compartment. Several investigations show an increase in the concentration of major plasma constituents during short, intense muscular exercise, primarily as a result of the decrease in plasma water (19,26,34,45,83). The rate and extent of the plasma volume shifts and lactate accumulation during and after physical stress, are related to the type and intensity of that stress. The response of the intravascular volume to running exercise also varies considerably among individual (26), as does the lactate response. Because fluid shifts will affect lactate concentrations, the pattern of lactate accumulation may therefore be influenced by fluid shifts. The purpose of this study was to eliminate any influence plasma volume changes may have on the construction of the lactate curve, by comparing lactate accumulation levels to absolute or total levels of lactate.

CHAPTER 2. LITERATURE REVIEW

2.1. LACTATE ACCUMULATION.

2.1.1. The classic concept of lactate accumulation.

It has been recognised since 1924 that lactate increases in the blood above a certain exercise level (3). This lactate threshold (LT) has also been called the anaerobic threshold (AT), to imply the onset of increased anaerobic glycolysis. Wasserman et.al.(88,90) defined the AT as the peak work rate or oxygen uptake at which aerobic metabolic processes can no longer meet the skeletal muscle requirements for ATP. As a consequence, as work is incremented above the AT, a progressive increase in anaerobic glycolysis must accompany aerobic metabolism to sustain adequate levels of ATP regeneration.

Normally there is a low concentration of lactate ([lactate]) in muscle and blood at rest, ie.~ 1mmol/kg wet muscle or litre of blood. At low exercise intensities, little or no change in [lactate] occurs (31,58). The source of this lactate is probably tissue with a low rate of oxygen utilisation (cornea, GIT, resting muscle, etc.) as well as erythrocytes, which do not possess mitochondria. As the exercise intensity increases, a point is reached where an increase in [lactate] in blood and muscle becomes evident (88).

The intensity of the exercise that elicits this rise in lactate concentration is variable and is influenced by a number of factors, including diet, physical fitness and type, intensity and duration of exercise (6,16,27,28,31,38,39,43, 47,58). The most rapidly accumulated and highest lactic acid levels are reached during exercise that can be sustained for 60 - 180 seconds ie. very high intensity (61). As the intensity of exercise decreases, thereby extending the work period, there is a corresponding decrease in both the rate of buildup and the final level of lactic acid.

This classic concept of lactate metabolism during exercise suggests that a deficit in oxygen uptake and delivery at the AT results in muscle anaerobiosis. When inadequate oxygen is available in the mitochondria, electron transport and rate of oxidative phosphorylation are slowed. This causes a fall in [NAD]/[NADH] and [ATP]/[ADP][Pi] of the mitochondria and cytoplasm. This in turn stimulates muscle glycogenolysis and glycolysis, with increased production of lactic acid and a consequent metabolic acidosis (71). It also causes changes in ventilatory and gas exchange dynamics.

The lactate/pyruvate ratio (L/P) of the contracting muscle in humans does not increase until the muscle PO_2 falls to critically low values (89,90). Muscle [lactate] remains at resting levels

until the muscle interstitial PO_2 falls to below 8mm Hg, at which point it increases sharply (9,18,89).

The role of lactate production is to "bank" some of the energy contained in the glucose molecule and transfer it to ADP for the production of ATP when required. Lactate production is considered by some therefore, as an "emergency method" for ATP production, or a supplement to the normal aerobic production of ATP.

Glycogen is used 18-19 fold more during anaerobic glycolysis compared to oxidative phosphorylation for the same ATP yield (23). Net production of ATP from the degradation of glucosyl units to lactate only releases 10% of the total energy stored in the glucose molecule. The rate of energy release however, can be the difference between a mediocre and high level performance. The relative importance of the aerobic and anaerobic components of metabolism to ATP production is related to the duration and intensity of the exercise (57).

2.1.2. Lactate accumulation not related to anaerobic metabolism.

The traditional view of lactate accumulation is being challenged with some investigators arguing that lactate production is a multifaceted process that is not always related to oxygen

availability, stating that the muscle always has enough oxygen during submaximal exercise (25). Connet et.al.(18), for example, showed that there were high levels of oxygen present in muscle mitochondria even when lactate was increased. Lactate production occurs as the result of the rapid breakdown of glycogen, and this can occur in the presence as well as the absence of oxygen (18,31).

A number of potential mechanisms have been suggested as being responsible for the sudden increase in blood lactate observed with increasing workload. Fibre recruitment (9,76), mitochondrial saturation (9,48) and hormonal factors (9,74) are among the more prominent variables thought to have a contributing role. Lactic acid production and output has been shown (18) not to be secondary to an inadequate supply of oxygen as mitochondrial NAD/NADH is highly oxidised while lactate output occurs.

Muscles contracting in isolation, in response to electrical stimulation, are not exposed to many of the variations in $[H^+]$, hormones and other metabolites that occur in exercise. Their metabolic response to contractions, in terms of lactic acid production and output, does not show a break or threshold. Lactate rises continuously - one or more of the variables of exercise must stimulate the muscle to produce more lactate as exercise progresses (14,74,75).

The results of tracer kinetic experiments have played an important role in elucidating these new theories (53). When metabolism is accelerated, as in exercise, lactic acid will inevitably be formed, however lactic acid does not necessarily accumulate at all levels of exercise. During light and moderate exercise, the energy demands of both trained and untrained subjects are adequately met by oxidative reactions. In biochemical terms, the ATP for muscular contraction is made available predominantly through energy generated by the oxidation of hydrogen. Any lactic acid formed is rapidly oxidised and the blood lactate level remains fairly stable even though oxygen consumption increases (57).

Brooks et.al.(8) studied the rates of lactate appearance (Ra) and disappearance (Rd) in humans during progressive increase in the intensity of exercise. During this form of exercise, Ra and Rd are linearly related to arterial lactate level and exponentially related to VO_2. These results indicated that the blood lactate inflection point during graded exercise was due to a greater rate of increase in Ra than in Rd. Stanley et.al. determined that lactate turnover exceeds glucose turnover by 32% when using [^{13}C] lactate and [^{14}C] glucose in humans during steady state cycling at 40% of VO_2 max (76).

2.1.3. Lactate accumulation during exercise.

Lactate production is an ongoing process that takes place in the resting individual, and during exercise is highly correlated to metabolic rate (4,46,58). Lactate does not necessarily accumulate at all levels of exercise. Immediately at the onset of light exercise, the production of lactate is increased but the lactate concentration does not change appreciably, therefore the rate of lactate degradation must also have increased (9,51,75). The increase in lactate concentration in blood during exercise is secondary to increased muscle lactate production. Blood lactate levels depend on the balance between rates of production and removal (50,87). An exercise intensity at 50%-60% of VO_2 max usually only elicits a rise in blood and muscle lactate in relatively unfit individuals. In trained runners there is little or no lactic acid accumulation until a running intensity is reached which requires 60%-90% of VO_2 max (28,57). Physiological responses to exercise are markedly altered at work rates where lactate increases (89).

An initial slow rise in lactate accumulation is followed by an exponential rise in lactate at faster run speeds (9,13,46). The precise reason for the rise in lactate during high intensity running is unclear, but involves more than O_2 availability (18,31). The work rate/exercise intensity where an excess of

lactate begins to accumulate during submaximal oxygen uptake has been defined as the lactate threshold (LT). Central to the evidence which supports the idea that the LT is the result of anaerobic metabolism, is the observation that blood lactate levels are lower in subjects who exercise while breathing hyperoxic gas mixtures (42). However, VO_2 does not increase, which is possibly an indication that hyperoxia increases lactate clearance. During exercise, the relative blood flow to gluconeogenic areas is decreased in favour of the working muscle, and relative lactate removal through gluconeogenesis is reduced (25). In the study by Hogan et al. (42) the mechanism of increased clearance is probably increased perfusion of tissues and organs capable of removing lactate, ie. the liver, which receives higher blood flow during exercise when hyperoxic gas mixtures are inspired (9,42).

During exercise, the main route of lactate removal is through oxidation (10,57). This conclusion is supported by results of studies on dogs (27,48), rats (25) and humans (76). A high VO_2 max and a good running economy (RE) are common characteristics of good and elite distance runners. However, the ability to perform at a high fraction of VO_2 max without an accumulation of lactic acid can determine success among runners who are relatively homogenous with respect to other aerobic factors (22,28,61). Runners with high LTs are capable of performances

that are superior to those of runners who have higher maximal aerobic capacities but lower LTs (28,58,79,80).

Acevedo investigated the physiological adaptations that resulted from increases in exercise training intensity in previously conditioned runners. He found that previously trained runners can increase training intensity to improve performance by lowering lactate production at the intensity at which they trained, despite no changes in VO_2 max (1). Eldridge (27) also found that with training there is improved efficiency of lactate removal during exercise which results in only minor blood concentration increases despite a large increase in production rate. This favourable response could be due to the athletes genetic endowment (type of muscle fibre), or specific local adaptations with training that would favour the production of less lactate (89), as well as a more rapid rate of removal at any particular exercise level (25). It is well documented that capillary density and the size and number of mitochondria increase with endurance training, as well as the concentration of various enzymes involved in aerobic metabolism (31,35,57). Such alterations enhance the cell's capacity to generate ATP aerobically.

2.1.4. Consequences of lactate accumulation.

The accumulation of protons caused by glycolysis have basically

two fates within the cell - they may be bound to negatively charged buffers, usually proteins (eg. haemoglobin), in the cell, or they may combine with pyruvate to form lactic acid.

Physiological responses to exercise are markedly altered at work rates where lactate increases. The increase in lactate concentration is accompanied by an equimolar increase in hydrogen ions which must be buffered immediately. In the plasma, this is accomplished almost completely by the bicarbonate buffering system (57,89). Vigorous exercise creates a great demand for buffering, and regulation of pH becomes progressively more difficult. At exercise intensities above 70% VO_2max, the rates of glycolysis and susequent production of H^+ are high. Following high intensity exercise to exhaustion, muscle pH can drop to values below 7.0 (65), which can cause nausea, headache and dizziness, as well as discomfort in the muscle groups involved in exercise (57).

This increased acidity will ultimately cause the breakdown of at least two components of muscle action (31,57,61). Firstly, the activity of the key glycolytic enzyme, phosphofructokinase, is reduced, which would reduce glycolysis, and subsequently interrupt the energy supply.

Secondly, inhibition of glycogenolysis can also occur at

different levels. During formation of cyclic-AMP, H^+ is a potent inhibitor of adenylcyclase. The conversion of phosphorylase b into the more active form phosphorylase a, by phosphorylase kinase, is inhibited by H^+. Phophorylase a will only accept the dianionic form of inorganic phosphate, which in the presence of excess H^+ is largely present as $H_2PO_4^-$, resulting in a decrease in substrate availability (65).

Thirdly, local muscular fatigue is generally attributed to a decrease in the number of active cross-bridges (41,65). Accumulated protons interfere with calcium regulation of the cross-bridge cycle by preventing calcium binding by troponin-C. Competitive inhibition by the accumulated protons with calcium for troponin binding has been suggested, or the process may involve a decreased Ca^{2+}-troponin affinity (31,61,65).

2.1.5. Source of lactate

2.1.5.1. Lactate accumulation not related to anaerobic metabolism.

Lactate accumulates in the blood during exercise secondary to an increase in muscle lactate, and depends on the balance between the rate of lactate appearance (Ra) and disappearance (Rd). Muscle lactate can increase because of various proposed mechanisms. (i) Glycolysis increases so rapidly that the

mitochondria cannot utilise pyruvate rapidly enough to prevent its elevation in the cytosol, which results in increased [lactate] as a result of the law of mass action (48,87).

(ii) The mitochondrial membrane shuttle which normally reoxidises reduced cytosolic NAD and transfers protons and electrons to mitochondrial co-enzymes for recombination with O_2, becomes rate limited. This causes a change in the cell redox state, thus converting pyruvate to lactate (78,89,90).

(iii) Lactate increases may be caused by the sequential recruitment of fibre types, in which Type 1 fibres (high mitochondrial capacity) are the predominant contracting fibres at low and moderate work rates, and Type 11 fibres (high glycolytic capacity) contract proportionally more at the higher work rates. No change in redox state is implied and therefore pyruvate must increase before or with lactate (2,77).

(iv) The terminal enzyme of the glycolytic pathway, lactate dehydrogenase (LDH), has the greatest catalytic activity (Vmax) of any of the glycolytic enzymes, which is several times greater than the combined activities of enzymes that provide alternate pathways of pyruvate metabolism. In addition, the K_m for the conversion of pyruvate to lactate is in the order of 0.08mM, which is sufficiently low to assure maximum stimulation of LDH

for the production of lactate (8, 23).

(v) Donovan and Brooks (25) postulated that increased blood [lactate] results from decreased lactate clearance. This could result from the reduced blood flow to the liver which occurs during exercise (8,25).

2.1.5.2. Lactate accumulation related to anaerobic metabolism. To distinguish between the concepts of lactate increases due to mass action or due to change in cell redox state, Wasserman et al (90) studied arterial lactate and pyruvate during an exercise test, and at the transition from exercise to rest. The [lactate], [pyruvate] and [lactate]/[pyruvate] ratio (L/P) were plotted against exercise VO_2. They found that at low work rates there was little change in any of the variables, and at moderate work rates [lactate] and L/P ratio increased at approximately the same VO_2.

[Pyruvate] also increased, but only at higher work rates and at a slower rate than [lactate]. On a log-log plot, the mean point where pyruvate started to increase was 31% above that of lactate. The slopes of both lactate and pyruvate log-log curves before this transition (rapid increase) point were above zero, but not significantly different from each other. The slope of the pyruvate curve after the transition was significantly less than the slope of the lactate curve, showing that pyruvate

accumulation accelerated less rapidly than that of lactate.

Wasserman et al concluded that small changes in [lactate] occur at low work rates and are proportional to changes in [pyruvate]. Major changes in [lactate] at and above the lactate threshold appear to be primarily determined by oxygen availability and changes in redox state rather than a mass action effect.

2.1.6. Fate of lactate.

Lactate production is an ongoing process that takes place in the resting individual and, during exercise is highly correlated to metabolic rate (10). It is the inevitable result of muscle glycogenolysis and glycolysis, and during exercise appears to serve at least two purposes, ie. (i) the maintenance of blood glucose through gluconeogenesis and (ii) the shuttling of oxidisable substrate, in the form of lactate, from areas of glycogenolysis (production) to areas of high cellular respiration (removal).

Virtually all tissues and cells can oxidise, at least to some extent, lactate to CO_2. The first major reaction is the oxidation to acetyl-CoA, which may enter numerous pathways (oxidation in the Krebs cycle, synthesis of fatty acids, cholesterol and ketone bodies). The major product in the body will vary with dietary and hormonal conditions, eg. it will be glucose in the starved

animal, glycogen and fatty acids in the fed one, ketone bodies in diabetes and CO_2 in exercise (50).

Most (75%) of lactate formed during sustained, steady-state exercise is removed by oxidation during exercise, and only a minor fraction is converted to glucose (8,9). As suggested from a-v difference measurements of lactate and lactate tracer across muscle, approximately half of the lactate formed in a working muscle bed is released into the venous circulation. The remaining lactate formed in the muscle and some removed from arterial circulation, are combusted within the muscle, and appear as CO_2 in the venous blood (10).

Of the lactate which appears in the blood, most will be removed and combusted by oxidative fibres in the active muscle bed and the heart (76,77). The shuttling of oxidisable substrate in the form of lactate from areas of high glycogenolytic rate to areas of high cellular respiration, through the interstitium and vasculature, appears to represent an important means by which substrate is distributed, metabolic "waste" is removed and the functions of various tissues are co-ordinated during exercise (10,25,31,66).

During recovery from sustained, exhausting exercise, most of the lactate accumulated during exercise will continue to be removed

by direct oxidation (10,25,31). As the muscle respiratory rate declines in recovery, lactate becomes the preferred substrate for hepatic gluconeogenesis. Practically all of the newly formed liver glucose will be released into the circulation to serve as a precursor for cardiac and skeletal muscle glycogen repletion (86).

Liver glycogen depots and muscle glycogen levels will not be completely restored until refeeding. During refeeding after fasting, a substantial amount of dietary glucose appears to bypass the liver, circulate to the skeletal muscle, and be converted to C-3 compounds (mainly lactate), which then recirculate to the liver and undergo glycogenesis. The muscle, therefore, plays a very important role in the carbohydrate metabolism of resting individuals (10,40).

After exercise, lactate will be favoured in comparison to other compounds, as a glycogenic precursor in the liver, but the mass of lactate, although elevated, is insufficient to restore liver glycogen to "normal" pre-exercise levels. This is because most of the lactate-carbon is disposed of as CO_2 during both exercise and recovery, therefore other gluconeogenic and glycogenic precursors must be utilised after exhausting exercise. The general consensus of studies which have utilised tracer lactate on resting or exercising animals or humans, support the

conclusion that oxidation is a major avenue of lactate disposal (8,10,25,48).

2.1.7. The influence of glycogen levels on lactate production.

The concentration of glycogen is easily altered by diet or exercise or a combination of the two. Changes in blood and muscle [lactate] induced by exercise are greatly influenced by the pre-exercise concentration of glycogen in the muscles that are engaged in the exercise. When glycogen is lowered by exercise or diet, there can be a reduction in maximal power and VO_2max (31,38,61). Under these conditions, lactate concentrations are below those attained when the muscle is well loaded with glycogen (31).

2.1.8. Measurement of the lactate response.

Although there is widespread use of the plasma lactate response, detection techniques vary considerably. Among the issues to be considered in obtaining accurate measures of blood lactate are: sampling site, timing of measurement, sample handling and preservation, and analytical methods used (4).

Blood samples are drawn from many sites - arteries (3,14,32, 40,88,89,90), capillaries (15) and most popularly, veins

(1,11,13,18,19,20,21,26,28,38,42,58). The most common field sampling site for lactate is the ear-lobe (39,59) or the finger tip, which yield arterialised capillary blood. In many exercise conditions, especially if the sampling site is cold, a good blood flow is difficult to obtain. When larger volumes of blood (more than 0,1-0,2 ml) or repeated measures are required, a venous catheter can be inserted at a convenient site. The catheter is kept free flowing by using heparinised saline which could possibly contaminate the blood sample and artificially lower the lactate level.

Whether the sample is obtained by lancing or via catheter, contamination by sweat can also be problematic as sweat lactate levels are considerably higher than blood lactate levels (5). Regardless of how the sample is obtained, continued lactate production within the red blood cell and clotting in the collected sample can still occur. In addition, the blood which picks up the lactate from the active muscle is diluted with blood from less active areas of the body. Simultaneously, the lactate is being removed from the blood at variable rates by the heart, liver, less active muscles and other organs. Lactate values determined from femoral blood samples may be 2x higher than values determined from cephalic or antecubital venous samples (33).

The protocol used to determine lactate accumulation may also have an effect (59,60,78), as lactate within the exercising body is far from a stable quantity, requiring that blood samples be handled carefully and consistently. As the lactate levels in the blood are dynamic, the timing of the blood draw is important, as is obtaining a good blood flow so that the sample can be obtained as quickly as possible (5).

The training status of the subject can also affect the lactate response curve due to increased metabolic rate, increased lactate removal rate and increased economy (biomechanical efficiency) in trained individuals (5,28,61,79,80). Other factors that could influence the lactate response could be any alteration in the environment, eg. temperature, altitude, track composition or pool length. The environment, time of day, warm-up and recovery should be standardised as much as possible to eliminate these influences.

2.1.9. The ventilatory threshold (VT).

Physical activity affects oxygen consumption and CO_2 production more than any other physiological stress. Ventilation increases to maintain proper alveolar gas concentrations to allow for the increased exchange of O_2 and CO_2. During light and moderate steady-state exercise, ventilation increases linearly with O_2 consumption and CO_2 production and averages 20-25 litres of air

for each litre of O_2 consumed. Ventilation is mainly increased by increasing tidal volume at low intensities, while at higher intensity exercise breathing frequency becomes more important. With this adjustment in ventilation there is complete aeration of the blood, with PO_2 and PCO_2 remaining at near resting level values (35,57,61).

The ratio of minute ventilation to oxygen consumption is the ventilatory equivalent and is usually ~25:1 during submaximal exercise. During more intense exercise, the increase in ventilation is disproportionate to the increase in oxygen consumption, resulting in the ventilatory equivalent increasing to between 35-40:1 (57).

It has been postulated that the LT can be determined during progressive exercise by observation of the point at which this disproportionate increase in ventilation occurs (67). The use of a respiratory event to characterise a metabolic event in muscle is based on several assumptions, one being that the change in muscle and blood lactate concentrations occur simultaneously (88). Some studies indicate that there is a time delay associated with lactate diffusion from the muscle, as well as retention of a substantial part of the lactate within the muscle (40) and diffusion hindrance for lactate above a certain concentration. Yeh et al.(92) found the non-invasive gas response had such a

large range of variability that they felt it was unsuitable for clinical work.

The use of progressive exercise in which frequent step increases in power output induce abrupt increases in ATP demand could compound the situation. In ramp work tests, VT is not affected by the slope of the ramp manoeuvre, whereas LT is greatly dependent on the ramp function (46). In progressive exercise, the threshold for muscle accumulation of lactate considerably preceeds the blood LT, which in turn preceeds the VT (13,32,62).

Green et al.(32) demonstrated that the ventilatory threshold occurred at a higher absolute and relative work intensity than the lactate threshold when using a progressive incremental protocol, which was in disagreement with previous research (23,88). The latter studies had concluded that the exercise level at which the blood lactate begins to increase corresponds closely to the exercise level at which the ventilation response during exercise demonstrates an abrupt increase in slope. Green explains the discrepancy by use of objective computerised multisegmental linear regression techniques to avoid subjective bias, a model previously validated by Orr et al (62).

Segal and Brooks (68) also observed significantly lower LT responses but significantly higher VT responses at given work

rates, an observation that was subsequently repeated by Hughes et al.(44) and Heigenhauser et al.(38). Hughes studied glycogen depleted subjects and found that the onset of blood lactate accumulation (OBLA) occurred at a significantly higher workload and $\%VO_2$ max than during exercise in the normal glycogen state. However the workload and $\%VO_2$ max at which VT occurred, shifted to a lesser workload during the glycogen-depleted state.

Hagberg et al.(36) studied the relationship between LT and VT in patients with McArdles syndrome. During incremental exercise tests, these patients, who lack the enzyme phosphorylase, had no blood lactate response. They did however, demonstrate a definite VT at a work rate which elicited approximately 70% VO_2max.

Simon (69) showed that ventilatory and lactate thresholds were coincidental in a trained subject, but not necessarily the same in untrained subjects. Acevedo (1) found that there can be a dissociation of the onset of blood lactate accumulation (OBLA) and the ventilatory threshold as a result of increased training intensity. Gaesser et al.(30) and Poole et al.(63) also observed that training resulted in a clear separation between VT and LT, ie LT shifted to a higher percentage of VO_2max but VT remained unchanged or the shift was less pronounced.

Part of the inconsistencies may be due to the terminology used to identify where the lactate threshold occurs and the many different protocols and methods used in evaluation of the LT and VT (54). If LT is taken as the exercise intensity at which the level of lactate increases to just above the resting value, this would mostly be lower than VT, where VT is taken as the exercise intensity at which the ventilation response shows an abrupt increase in slope.

2.1.10. Lactate and catecholamines.

Exercise also affects the levels of circulating catecholamines, with the magnitude of the increase being related to the intensity of the effort as well as the duration of the exercise (57,87). Other factors also determine catecholamine response to exercise, including age (plasma catecholamine secretion is greater in older subjects at the same exercise intensity) and gender (there is greater adrenalin secretion in males compared with females who perform the same relative intensity of exercise) (29,57).

Sympathetic activity affects many factors, including blood flow distribution, enhanced cardiac contractility, substrate mobilisation, liver glycogenolysis and adipose tissue lipolysis - all of which are beneficial to the exercise response.

Beta-adrenergic agonists (eg. adrenalin) increase muscle lactate output during contractions and have a major effect in determining the magnitude of the lactate response (8). Adrenalin infused into the bloodstream in a progressive manner causes an elevation of blood [lactate] (74). Results of studies on animals implicate beta-adrenergic stimulation of muscle glycogenolysis as a cause of lactate formation (8,48,73). A high correlation (0.92) was found between tracer measured blood lactate appearance and the arterial adrenalin level in running rats (64). Results obtained on exercising humans support the conclusions reached on the basis of animal experiments (56).

During progressive exercise adrenalin and noradrenalin concentrations increase. They rise slightly up to approximately 50% of VO_2max. Adrenalin concentration increases sharply during exercise as VO_2 exceeds 50% of VO_2max (74). This increase causes an increase in lactate release in the working muscle and reduces uptake by the liver (87). Both working and inactive muscle contribute to lactate exchange during exercise (72,81). Adrenalin exerts an acute and enhancing effect on muscular glycogenolysis.

Via the beta-adrenergic receptors, the catecholamines set into action a series of cascading reactions that lead to the action of phosphorylase a, the regulating enzyme for glycogenolysis. During muscular contractions, increases in cytoplasmic Ca^{2+}

concentration, as the result of release from the sarcoplasmic reticulum, have also been shown to activate phosphorylase a. Although Ca^{2+} release contributes to the breakdown of glycogen during muscular activity, this appears to be a transient response (48,91). In the transition from rest to exercise, the Ca^{2+} mediated control of muscle glycogenolysis predominates, however, once the catecholamines have been activated and released, control of glycogenolysis shifts to hormonal control.

Lactate release from skeletal muscle is mediated by beta-adrenergic agonists, whereas alpha-adrenergic agonists do not stimulate lactate production (8,48,73). The blockade of beta-adrenergic receptors in exercising dogs had two striking effects, ie. the inhibition of lactate production and the increase of glucose turnover (48). Inhibition of Ra of lactate was much more profound when the blocker was infused steadily into the running dog than when administered in a single injection prior to exercise (48). Beta-blockade inhibits the exercise-induced muscle glycogenolysis, resulting in an increase in the ability of the working muscle to take up glucose, thus the bulk of lactate arises from plasma glucose and not from muscle glycogen (18,48).

The rate of glycogenolysis in the working muscle is therefore under dual control; an intracellular metabolic control and a hormonal control that involves beta-receptors in the muscle.

2.2. BODY FLUIDS

2.2.1. The effect of body fluid shifts on the concentration of plasma constituents.

Van Beaumont et al. (82,83) evaluated the effect that haemoconcentration had on the concentration of plasma proteins and electrolytes during maximal exercise. They felt that conclusions concerning physiological homeostatic mechanisms, based solely on changes in plasma solute concentrations, could lead to erroneous interpretations. It has been known for some time that during short, intense muscular activity, fluid is lost from the vascular compartment (19,26,34,37,55,70).

Proportional changes in plasma volume can be calculated fairly accurately from changes in haematocrit alone, especially at submaximal work rates and in a cool enviroment (82). Van Beaumont et al. reported that, although the red cell mass remains constant in this situation, the volume of human red blood cells can increase, decrease or remain constant during physical stress depending on the interactions of plasma osmolality and blood pH (84). In order to eliminate any error which can occur because of changes in the red blood cell volume, both haematocrit (Hct) and haemoglobin (Hb) values should be used (19,20,37,84). A study by Kanstrup et.al. (49) emphasised the total amount of Hb rather

than the Hb concentration as being important for high O_2 uptake and physical performance.

The acid-base balance in the forearm venous blood is only influenced to a minor extent by submaximal leg exercises, with non-significant changes at work levels less than 55% of VO_2max, and small decreases at work loads between 55% and 75% of VO_2max. With maximal exercise (85-100% of VO_2max), the blood pH can decrease to below 7.10 (65). It seems that the greater the acidification of the blood, the greater the chance for red cells to increase in volume (20,85). In many cases, the erythrocyte continues to swell after termination of short-term, maximal exercise. Possibly, under severe lactacidotic conditions, the entry of lactic acid into the erythrocytes is fast enough to account for the delayed swelling after exercise, especially since the highest lactate concentrations are found after short, maximal exercise (84).

Volume changes in the human erythrocyte can only be properly assessed when both osmotic and acid-base parameters are considered simultaneously. The extent and the rate of plasma volume shifts are related to the type and intensity of that stress (84). The response of the intravascular volume to running exercise varies considerably among individuals (26).

The expected concentrations of a plasma constituent following a fluid shift can be calculated from the following equations:

(i) Using haematocrit only:

$$[C_E] = [Hct_2(100 - Hct_1)]\ C_1 \ /\ [\ Hct_1(100 - Hct_2)] \quad (83)$$

(ii) Using haematocrit and haemoglobin:

$$[C_E] = [Hb_2(100 - Hct_1)]\ C_1 \ /\ [\ Hb_1(100 - Hct_2)] \quad (84)$$

where C_E = Expected concentration if the change in concentration is the result of a change in solvent only.

C_1 = Plasma lactate concentration at baseline (first workload in this study)

Hct_1 = Haematocrit value at baseline

Hct_2 = Haematocrit value at subsequent workloads

Hb_1 = Haemoglobin value at baseline

Hb_2 = Haemoglobin value at subsequent workloads

The actual gain, or loss, of the solute is the difference between the expected and the measured concentrations.

2.2.2. Factors influencing the change in plasma volume.

Percent change in plasma volume (ΔPV%) can be calculated from the following equations:

(i) Using haematocrit values only:

$$\Delta PV\% = [100/(100 - Hct_1)] \times 100(Hct_1 - Hct_2)/Hct_2 \quad (82,85)$$

(ii) Using haematocrit and haemoglobin values:

$$\Delta\% PV = 100\ [Hb_1(100 - Hct_2)/Hb_2(100 - Hct_1)] - 100 \quad (84)$$

Maximal work load, maximal oxygen consumption, as well as variations in blood pressures and capillary diffusibility contribute to the range of loss in plasma fluid during exercise. Haemoconcentration is apparent shortly after the start of muscular activity, with the largest change in plasma volume occurring during the first 4 - 6 minutes of exercise (34). After the termination of the physical activity, restoration of the plasma volume to pre-exercise level takes approximately 60 minutes (17,37).

It appears that plasma volume shifts during submaximal exercise are due mainly to variations in hydrostatic pressure and intracellular osmolality (34). With augmented work stress, incremental plasma volume losses may be related to proportionally

greater increases in capillary pressure and osmolarity in the active muscles (55).

CHAPTER 3. MATERIALS AND METHODS

3.1. Subjects

Eleven healthy, non-smoking subjects, (8 males, 3 females), volunteered to participate in the study. Ten of the subjects were participating in competitive road running and one in a high intensity aerobics programme. The physical characteristics of the subjects are shown in Table 1.

3.2. Exercise testing.

3.2.1. Maximal oxygen consumption.

Initially we measured each subject's maximal oxygen uptake (VO_2 max) using an intermittent, incremental protocol, on a motorised treadmill (E10, Powerjog, Birmingham, England). During all exercise, oxygen consumption was measured using a precalibrated, online system (Oxycon 4, Mijnhardt, Bunnik, Holland). The end point was taken when the difference between the VO_2's on two successive workload increments (1% change in elevation), was less than 1,0 ml/min.kg. Subjects received verbal encouragement during the test and were required to sustain exercise for a three minute period.

3.2.2. Running economy.

The running economy of each subject was also determined, at 12.0 km/hr and 0% elevation, on the treadmill (Table 2).

Running Economy (RE) was calculated as:

$$RE = (VO_2 - \text{Rest } VO_2) / \text{Velocity}$$

3.2.3. Lactate measurement.

After a week's recovery, we determined the lactate curve of each subject, on three different days, using a graded continuous exercise protocol, on the treadmill (Table 3). Measurement of the three lactate curves were carried out within a two week period to minimise changes due to training effects. Subjects were requested to maintain their usual dietary habits, leisure time activities and training programmes during the testing period. We measured the lactate curve of each subject on three different occasions to test the reproducibility of the lactate response during exercise.

3.3. Procedure.

3.3.1. Blood collection.

Venous blood samples were collected into evacuated EDTA tubes, via a winged infusion set inserted into a forearm vein, and kept patent using a heparinised saline solution. To reduce the possibility of contamination of the blood sample by the heparinised saline solution, the first portion of the sample drawn was discarded. The winged infusion set was inserted while the subject was seated. The subject then stood on the treadmill while the metabolic cart was calibrated, the ECG leads were

connected, the Hans Rudolph helmet was adjusted and the two-way non-rebreathing valve was positioned. The period of standing exceeded 20 minutes to allow postural equilibration of body fluids.

3.3.2. Lactate determination.

The subject commenced exercising at 5.5km/hr and 0% elevation. The first blood sample was collected after 2,5 minutes of walking, and this sample was used to measure the baseline Hct, Hb and lactate levels. The workload was increased without a break in exercise, and all subsequent measurements were made during the third minute of graded exercise, until an oxygen consumption greater than 90% of VO_2 max was reached. Exercise heart rate was measured using a three lead subcostal ECG recording. The total period of exercise lasted between 24 to 30 minutes.

3.3.3. Analytical methods.

The blood samples from each workload were used to measure haematocrit, haemoglobin and plasma lactate concentration. Haematocrit was measured immediately, in duplicate, using a microhaematocrit centrifuge, for all eleven subjects.

Haemoglobin was measured using a Technicon H3 Coulter Counter for seven of the subjects, only during one of the three lactate curve determinations. The plasma used to measure lactate was stored at

-20°C and analysed 2-4 weeks after collection using an enzymatic technique (Boehringer Mannheim).

3.4. Curve construction

3.4.1. Lactate threshold (LT).

The lactate curve was constructed by relating lactate concentration to steady state oxygen consumption, expressed as percent VO_2 max. The lactate threshold (LT) was determined individually for each subject, for each test. We utilised a computer program which fitted two straight lines, with slopes b1 and b2, which intersect at x = LT and y = a. [y = a + b1(x - LT) where x < or = LT; y = a + b2(x - LT) where x > LT]. The mean of the three determinations for each subject was used for statistical analysis.

3.4.2. Exponential model.

Because of the controversy regarding the existence of a lactate threshold (7,8,64), the lactate curve was also constructed by fitting a least squares regression exponential model to individual data (Tablecurve-Jandel Scientific, 1974). The form of these curves were expressed as y = a + bexp(-x/c). A separate curve was constructed for each test, and the mean for each subject was used for comparisons.

3.4.3. Influence of fluid shifts.

The influence of fluid shifts on the lactate concentration, during the continuous incremental exercise, was assessed by calculating the anticipated or expected lactate concentration if change in lactate concentration is the result only of fluid shifts. The accumulated lactate concentration corrected for body fluid shifts was calculated as:

Accumulated [lactate] = Measured [lactate] - Expected [lactate]

EQUATIONS FOR DETERMINATION OF EXPECTED [LACTATE].

1) Using haematocrit only:

$$C_E = C_1[\ Hct_2\ (100 - Hct_1)\ ]/\ [\ Hct_1\ (100 - Hct_2)] \quad (83)$$

2) Using haematocrit and haemoglobin:

$$C_E = C_1\ [\ Hb_2\ (100 - Hct_1)]/\ [\ Hb_1\ (100 - Hct_2)\ ] \quad (84)$$

where: C_1 = plasma lactate concentration at the first workload.

Hb_1 = the haemoglobin value at the first workload

Hb_2 = the haemoglobin value at each subsequent workload.

Hct_1 = the haematocrit value at the first workload

Hct_2 = the haematocrit value at each subsequent workload.

The lactate concentration at each workload was then converted to the accumulated lactate corrected for body fluid shifts,

according to the change in plasma volume (PV), using either haematocrit values alone (n=11, repeated three times) or haematocrit and haemoglobin values (n=7), as described by Van Beaumont et.al.(82,83,84,85), and Dill et.al.(24).

EQUATIONS FOR THE CALCULATION OF CHANGES IN PLASMA VOLUME

(i) Using haematocrit only:

$$\% \Delta PV = 100/(100-Hct_1) \times 100(Hct_1-Hct_2)/Hct_2 \quad (82,85)$$

(ii) Using haematocrit and haemoglobin:

$$\% \Delta PV = 100\ [Hb_1(100-Hct_2)/Hb_2(100-Hct_1)] - 100 \quad (84)$$

The accumulated lactate concentrations corrected for body fluid shifts were used to reconstruct the individual curves using the two different models, ie. lactate threshold and exponential fit. The lactate threshold on the one hand and the $\%VO_2max$ at a lactate concentration of 4mmol/l on the exponential plot on the other hand, were compared before and after conversion of the lactate concentrations from absolute (total) levels to accumulated levels corrected for body fluid shifts. Once again, the curves were constructed separately for each test, and the mean for each subject was used for statistical analysis. Differences between values measured before and after were compared statistically using the student t-test for matched dependent data. The null hypothesis was rejected at the 5% level.

Chapter 4. RESULTS

4.1. Subjects.

The mean of individual measurements of body mass, age and height are presented in Table 1, with the mean responses to maximal exercise and running economy shown in Table 2. All subjects were in good health with a mean body mass index of 22,8.

Table 1. Physical characteristics of subjects.

	AGE (yrs)	HEIGHT (cm)	MASS (kg)	BODY MASS INDEX (Kg/m^2)
MEAN	33.45	170.7	66.86	22.8
RANGE	24-43	159-180	48.0-86.5	19.0-28.4
± SD	5.7	7.2	11.92	2.8

Table 2: Physiological variables during maximal exercise

	VO_2 max l/min	VO_2 max ml/kg.min	RE mlO_2/kg.km	HR(max) beats/min
Mean	3.64	55.08	167.8	184
Range	2.66 - 4.49	46.5 - 69.7	157.3 - 187.8	169 - 198
+/- SD	0.58	6.9	10.83	9.96

where RE = Running Economy

Table 3: Protocol used for the determination of the lactate curve.

TIME (minutes)	SPEED (km/hr)	ELEVATION (%)
0 - 3	5.5	0.0
3 - 6	5.5	6.0
6 - 9	5.5	12.0
9 - 12	5.5	15.0
12 - 15	5.5	18.0
15 - 18	6.0	18.0
18 - 21	6.5	18.0
21 - 24	7.0	18.0

4.2. Percent change in plasma volume.

Changes in plasma volume were calculated for each workload of the lactate determination, for each subject, using either Hct values on their own or by combining Hct and Hb values. An example, for

subject PP is presented in Table 4. The largest change in plasma volume, when using Hct only, occurred during the first nine minutes of exercise, which has also been described in previous studies (34). When using both Hct and Hb, the largest change occurred during the highest workload, ie. after 24 minutes of exercise and above 80% of the subject's VO_2max.

Table 4: Percentage plasma volume changes for subject PP.

TOTAL EXERCISE TIME (min)	%VO_2 max	% PLASMA VOLUME CHANGE USING Hct	%PLASMA VOLUME CHANGE USING Hct & Hb
2.5	23.89	-7.716	-8.106
5.5	35.22	-6.974	-9.814
8.5	47.38	-11.338	-11.392
11.5	52.73	-7.518	-9.144
14.5	60.65	-5.090	-6.869
17.5	65.03	-3.166	-10.095
20.5	71.95	-7.344	-11.152
23.5	79.81	-9.546	-10.523
26.5	87.63	-8.818	-15.404

There are slight differences in the %change in plasma volume when using the 2 methods (ie. Hct only or Hct and Hb), although no definite trend was apparent (figure 1). The curves are similar, except for the last workload, where the subject was working at

above 80% of VO_2max. This could be due to changes in the volume of the red blood cell at maximal exercise, as described in previous studies (20,85).

There were large individual variations in the maximum percentage change in plasma volume, but such individual variations have frequently been observed following exercise and thermal dehydration (21). When using Hct values to determine the percentage change in PV, the maximum ΔPV% varied from -3,23% to -18,57% with the mean maximum ΔPV% = -9,70 +\- 4,68% (n=11). When using both Hct and Hb, the change in PV ranged from -3,83% to -15,40% with the mean maximum ΔPV% = -8,21 +\- 3,80% (n=7).

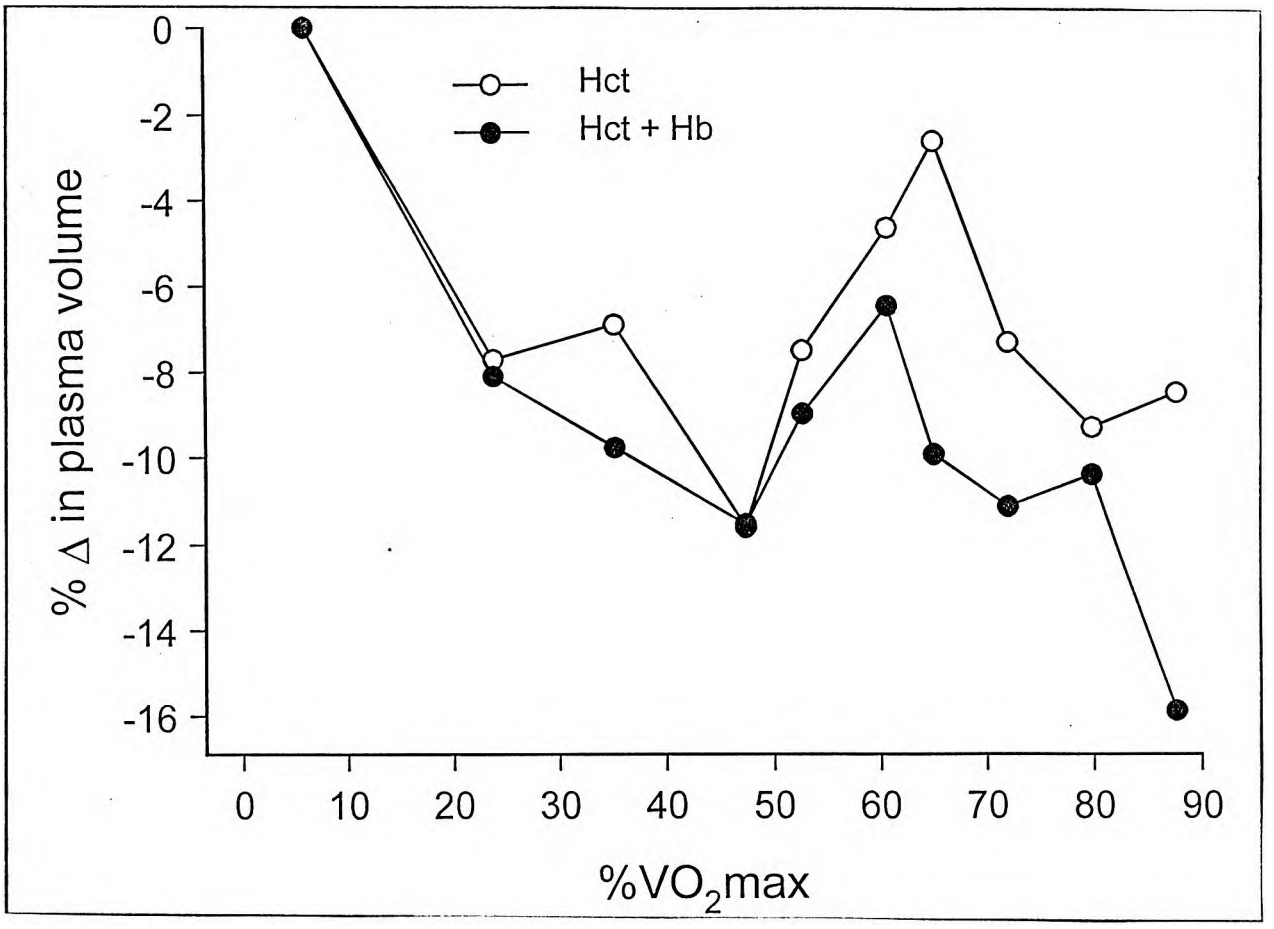

Figure 1: Comparison of %changes in plasma volume using Hct values alone or a combination of Hct and Hb values for subject PP.

4.3. The lactate threshold.

4.3.1. Reproducibility.

In each subject, the lactate threshold was determined separately for each repetition, for both the unconverted and converted data. Figure 2 compares the three unconverted repetitions for subject HG, showing good reproducibility with a coefficient of variation (%CV) of 2.52%. (Average coefficient of variation for the group = 2.68%). The mean of the three LT determinations was used for statistical analysis. Figure 3 compares the data converted using Hct values, and once again the three curves show good reproducibility with %CV = 3.63, (average coefficient of variation for the group = 3.60%).

Lactate threshold data obtained from subjects before and after adjusting for body fluid shifts, using haematocrit only, are presented in Table 5. These LT values are the mean of the three repeat measurements for each subject.

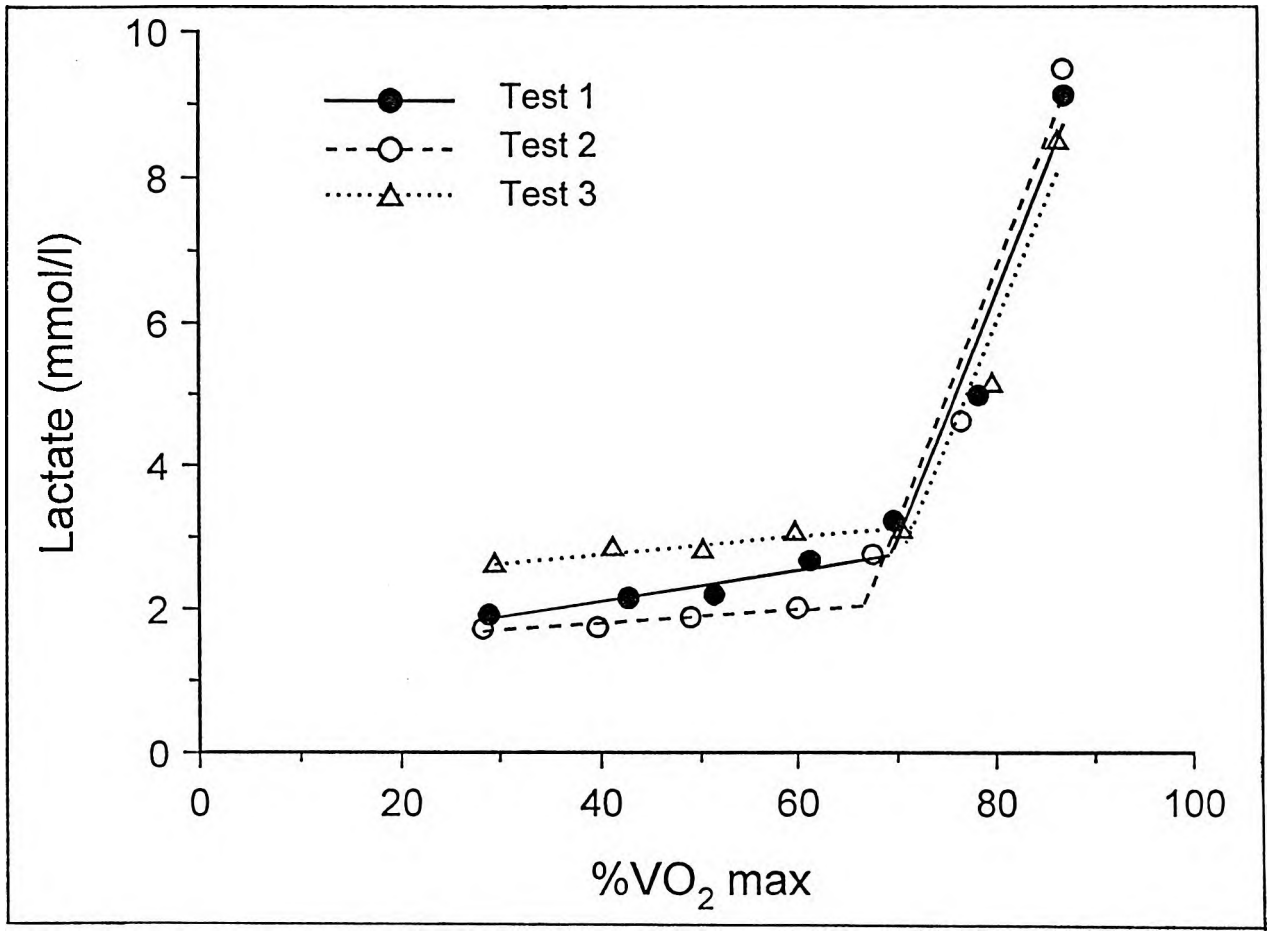

Figure 2: The lactate threshold - a comparison of three determinations for subject HG.

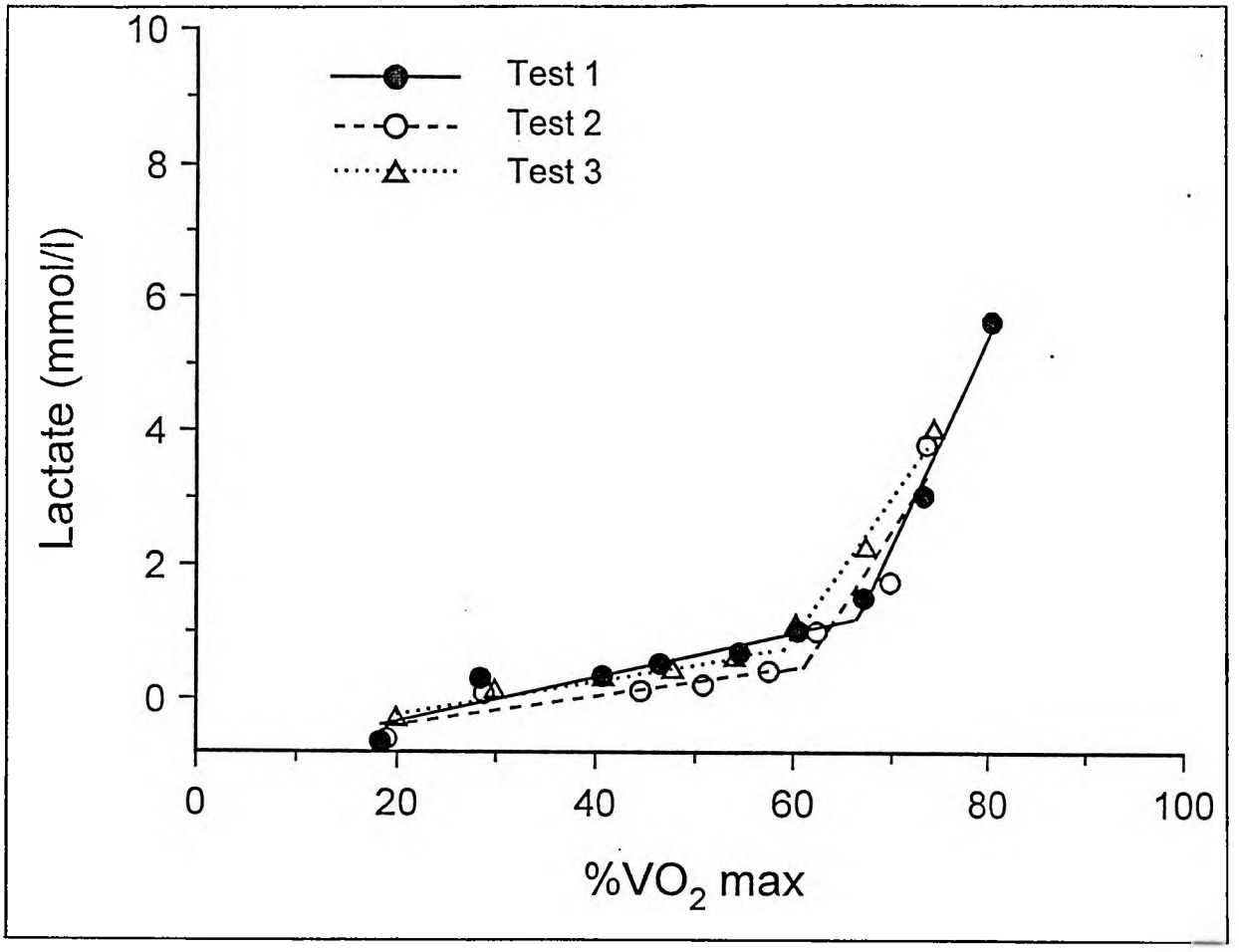

Figure 3: The lactate threshold - a comparison of three determinations after adjusting for body fluid shifts in subject HG.

Table 5: The Lactate Threshold - differences when adjusting with haematocrit.

Subject	LT unconverted $\%VO_2$ max	LT converted using Hct $\%VO_2$max	Difference
AC	82.81	83.62	0.81
ZY	57.04	58.57	1.53
PP	68.19	68.82	0.63
TP	76.13	75.63	-O.50
DD	72.02	72.14	0.12
HG	74.02	74.50	0.48
DS	73.42	73.69	0.27
OC	72.84	73.01	0.17
D	60.04	60.50	0.46
CO	70.39	72.23	1.84
RD	59.59	64.99	5.40
MEAN	69.68	70.70	1.02
SD	7.87	7.15	1.59

4.3.2. Adjusting with Hct alone compared to adjusting with Hct combined with Hb.

Converting to the accumulated lactate concentration and correcting for plasma volume shifts using haematocrit only

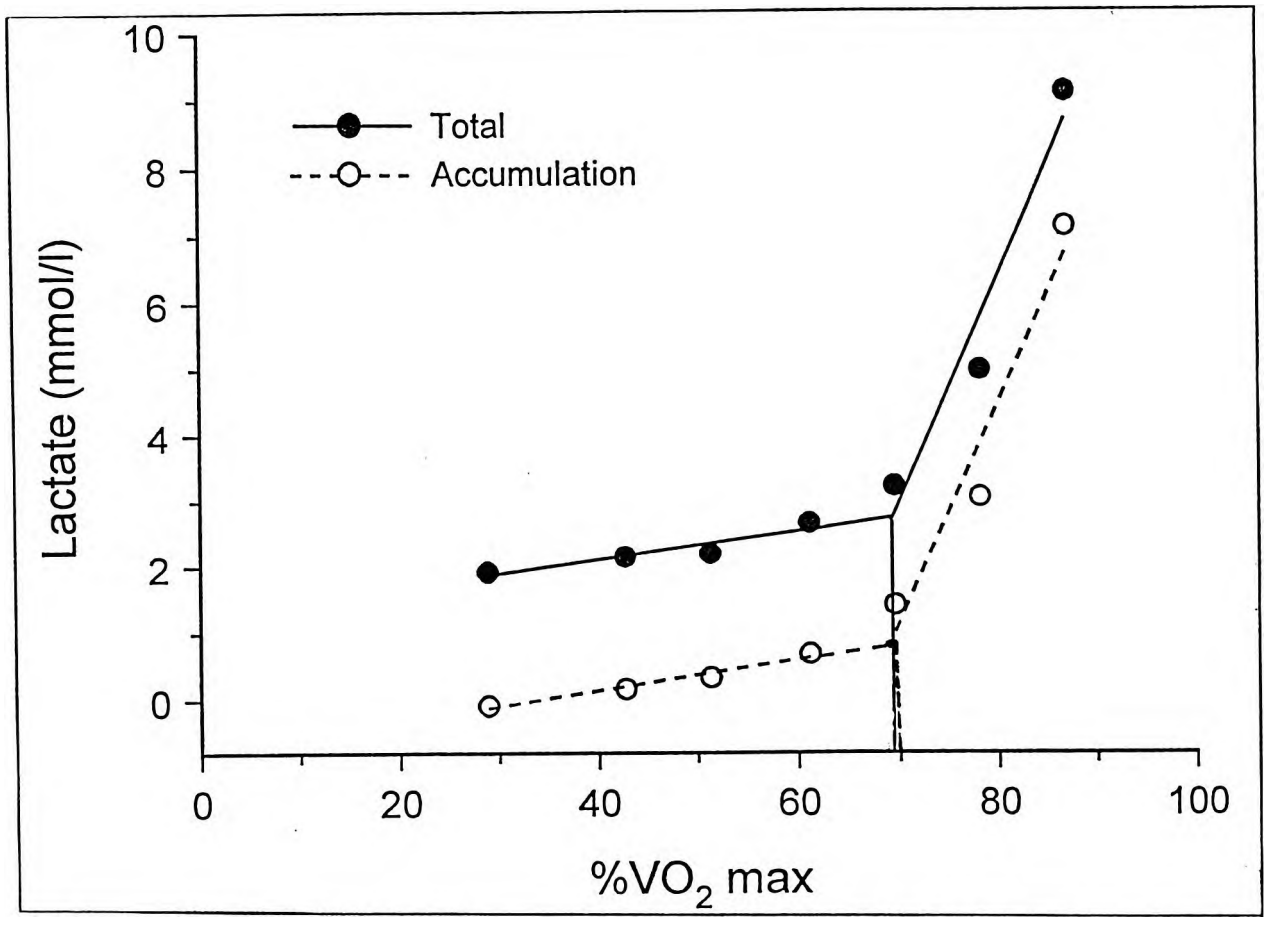

Figure 4: The lactate threshold - a comparison of $\%VO_2max$ at LT, before and after converting from total lactate to accumulated lactate corrected for body fluid shifts using Hct (Test 1).

shifted the lactate threshold slightly to the right, ie. to a marginally higher value of VO_2 max (figure 4). The average difference in LT, when adjusting with Hct only, was 1.02 of $\%VO_2$ max, which represents a 1.46% change (Table 5).

When adjusting with Hct and Hb (Table 6), the average change was 0.355 of $\%VO_2$ max, which represents a 0.5% change (figure 5). The shift in both cases was not significant. When comparing the two different curves, there is not much difference between the LT result for data adjusted with Hct and that adjusted with Hct and Hb (figure 6). This indicates that although the latter is considered the more accurate approach, adjusting for body fluid shifts using Hct only, does provide an acceptable approximation. The comparison of %changes in plasma volume using Hct alone or a combination of Hct and Hb values for subject PP (figure 1) also shows there is little difference between the methods of determining body fluid shifts.

Table 6: The Lactate Threshold - Differences when adjusting with Haemoglobin and Haematocrit.

Subject	Lactate Threshold $\%VO_2max$	LT adj with Hct and Hb	Difference
AC	82.81	83.54	0.73
ZY	57.04	57.77	0.73
PP	68.19	68.77	0.58
TP	76.13	75.51	-0.62
DD	72.02	72.63	0.61
HG	74.02	74.23	0.21
DS	73.42	73.67	0.25
MEAN	71.95	72.30	0.36
SD	7.94	7.81	0.48

Table 7: Comparison of the LT when adjusting with Hct alone and when using a combination of Hct and Hb.

Subject	LT adjusted with Hct	LT adjusted with Hct & Hb	Difference
AC	83.62	83.54	0.08
ZY	58.57	57.77	0.80
PP	68.82	68.77	0.05
TP	75.63	75.51	0.12
DD	72.14	72.63	-0.49
HG	74.50	74.23	0.27
DS	73.69	73.67	0.02
MEAN	72.42	72.30	0.12
SD	7.60	7.81	0.38
t			0.836

Difference not statistically significant.

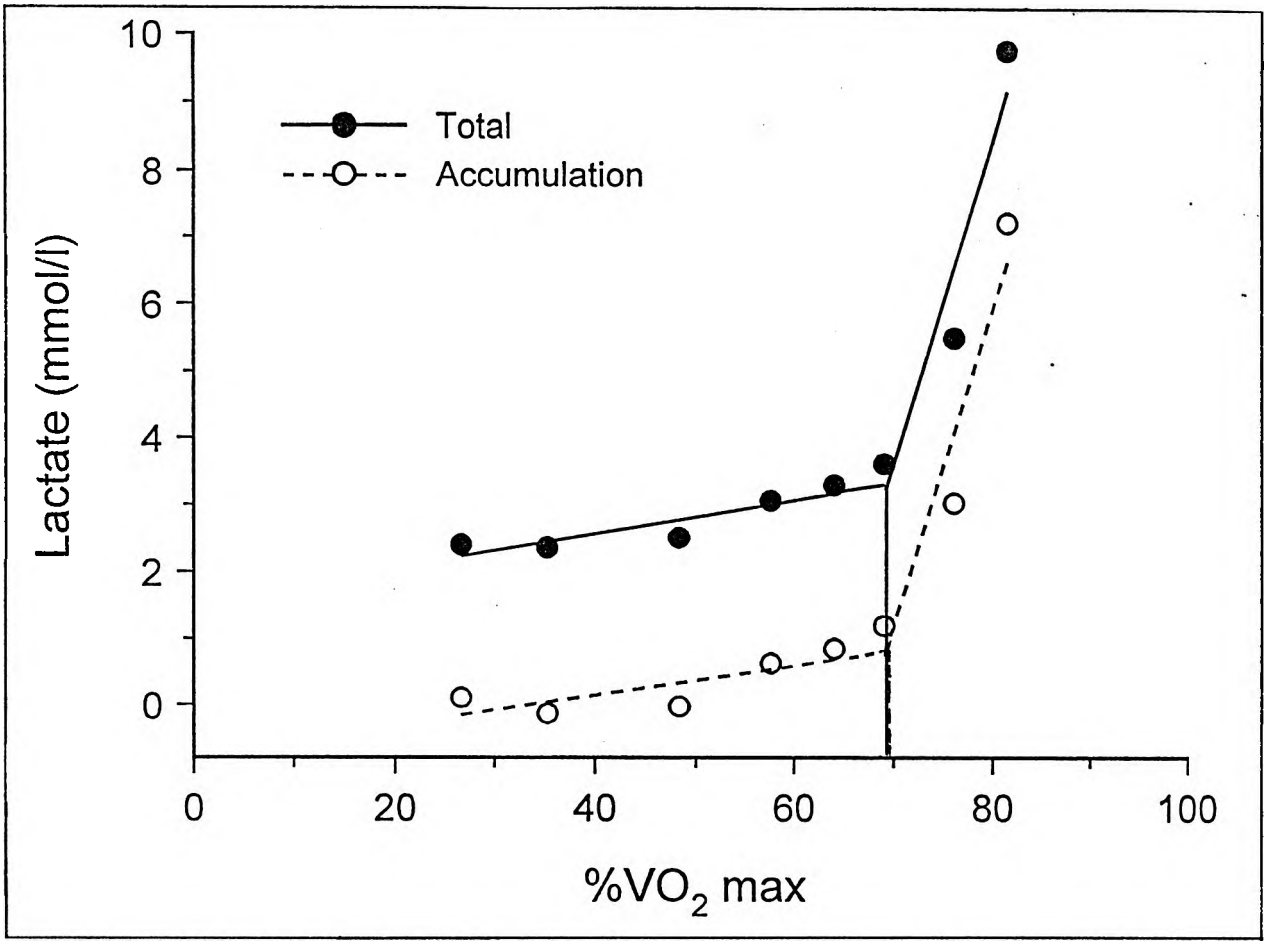

Figure 5: The lactate threshold - a comparison of VO_2max at LT, before and after converting from total lactate to accumulated lactate, adjusted for body fluid shifts using Hct and Hb values in subject DS.

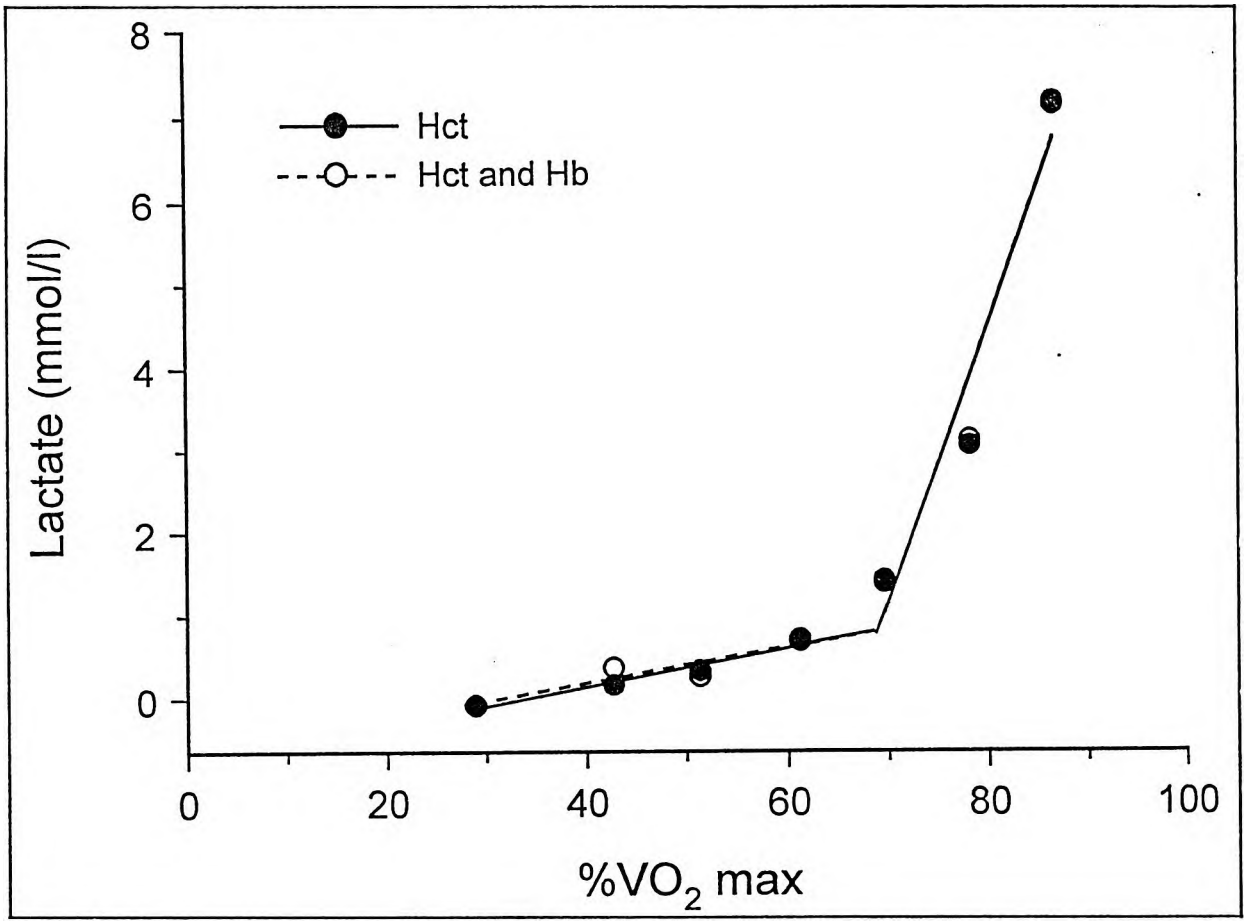

Figure 6: The lactate threshold - a comparison of curves adjusted for body fluid shifts with Hct and those adjusted with both Hct and Hb, in subject HG.

4.3.3. Total lactate compared to accumulated lactate adjusted for body fluid shifts.

When comparing total lactate to the adjusted accumulated lactate, subject TP, who suffers from Thallasaemia had the only intercept that shifted to the left (Tables 5 and 6). If these data were omitted, the differences increased to 1.17 of $\%VO_2$ max or 1,65% (Hct) and 0.519 of $\%VO_2$ max or 0,72% (Hb & Hct). The changes then become significant (Table 8).

Table 8: Difference in lactate turnpoint between total lactate and adjusted accumulated lactate when omitting the subject with Thallasaemia.

	Difference in LT - Adj. using Hct	Difference in LT - Adj using Hct & Hb
MEAN	1.171	0.518
STD. DEVIATION	1.591	0.232
STD. ERROR	0.503	0.0947
t	2.328	5.470
	$p < 0.05$	$p < 0.002$

4.4. Exponential model.

4.4.1. Reproducibility.

Due to the controversy surrounding the existence of a lactate threshold, an exponential curve was fitted to each subject's data. Once again, a curve was fitted to the data for each of the three repetitions. An excellent fit was obtained when using the original data (r = 0.989 +/- 0.018), as well as for the data converted by adjusting with haematocrit (r = 0.985 +/- 0.024) or by adjusting with haematocrit and haemoglobin (r = 0.988 +/- 0.027). A comparison of the three curves using unconverted data shows excellent reproducibility (figure 7), as does a comparison of the three curves using the converted data (figure 8).

The $\%VO_2max$ at a lactate concentration of 4 mmol/l was determined for each set of data, as a measure of the position of the curve (figure 9). Using the $\%VO_2max$ values at the 4mmol/l lactate concentration, the coefficient of variation for the three repetitions of the original data for subject ZY was 2,26%, and the average %CV for the group was 1,99%. The %CV for the three repetitions of the converted data for subject ZY was 1,20% and for the group the average %CV was 3,03%. The mean value for the three repeat lactate curve determinations was used for statistical analysis.

4.4.2. Comparison of total lactate and accumulated lactate adjusted for the shift in body fluids.

As was observed when using the lactate turnpoint, the shift in the curve was down and to the right, ie. to a higher % of VO_2 max. In this case, however, the shift was more pronounced. The differences between the $\%VO_2max$ at a lactate concentration of 4mmol/l, before and after adjustment, were compared using the t-test for paired observations. The shift was found to be significant ($p < 0,001$) in both cases (Hct only : $t = 14,375$; and Hct and Hb : $t = 9,106$). The data is presented in Tables 9 and 10.

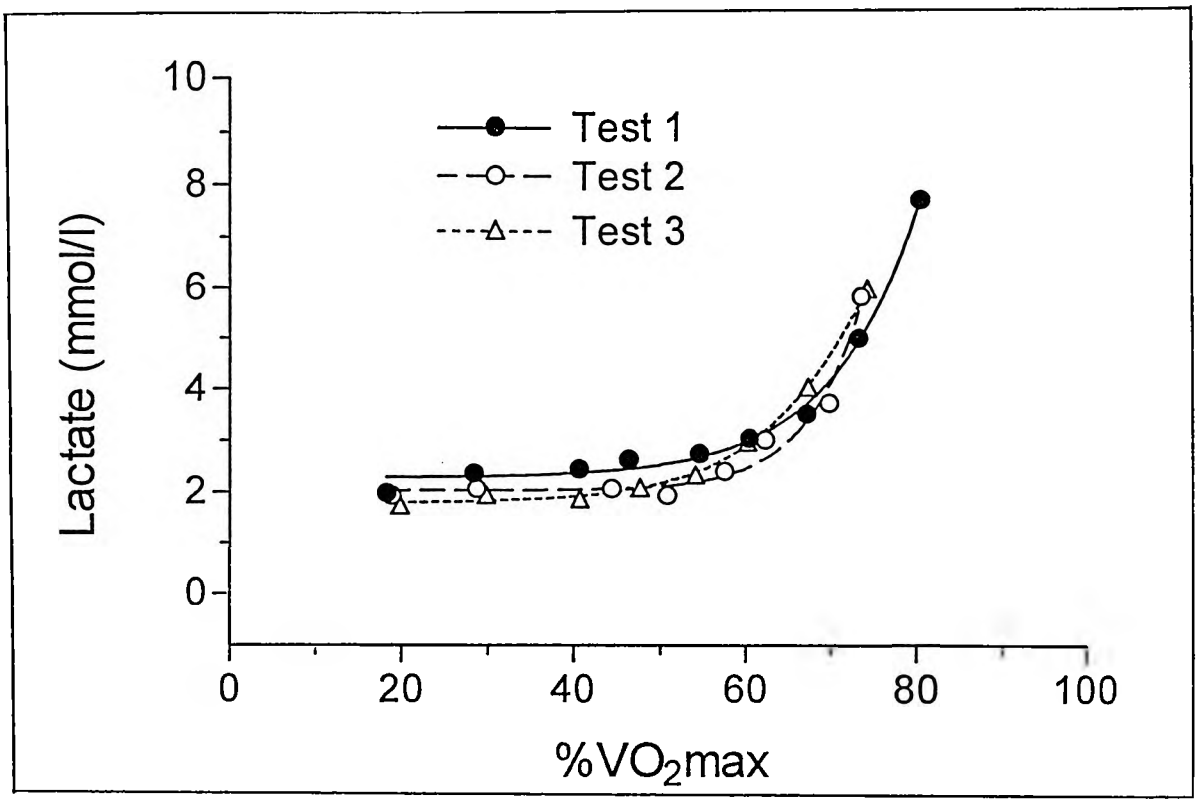

Figure 7: The exponential fit for subject ZY - a comparison of three repetitions for total lactate concentrations.

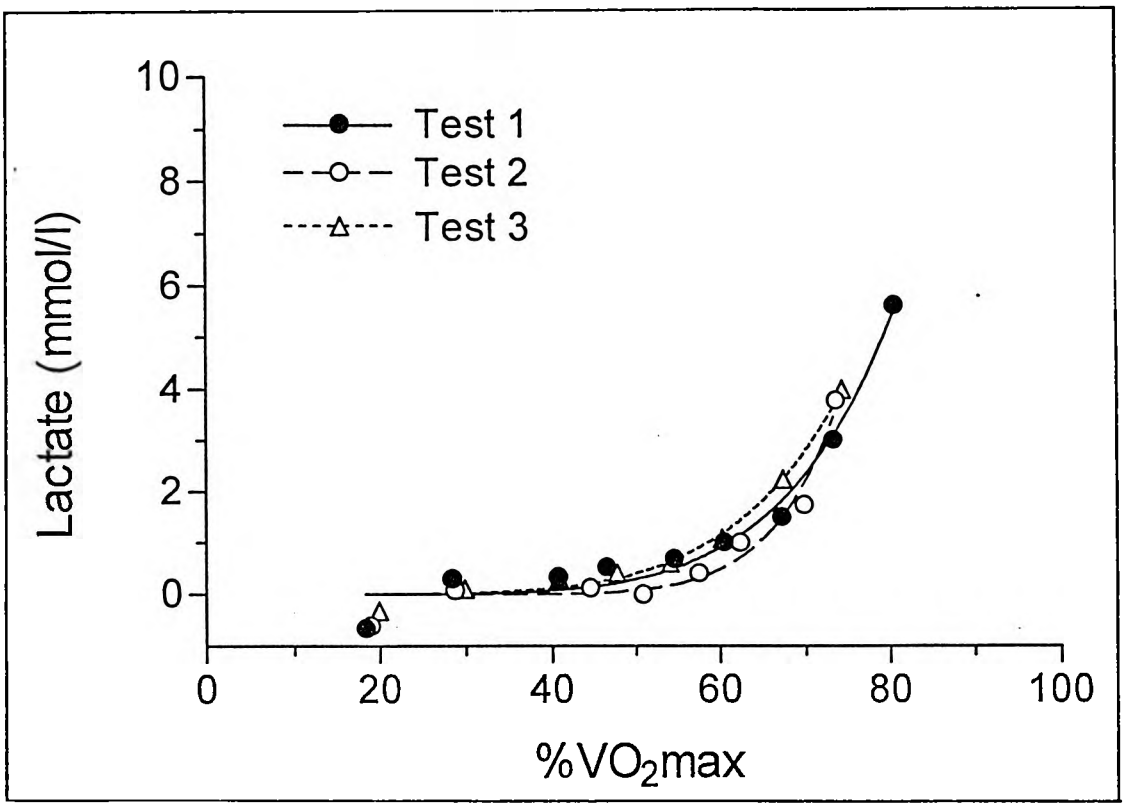

Figure 8: The exponential fit - a comparison of three repetitions after converting to accumulated lactate and adjusting for body fluid shifts using Hct only in subject ZY.

Table 9: Comparison of the %VO_2max at [lactate] of 4mmol/l before and after converting to accumulated lactate and adjusting with Hct, when using the exponential model.

SUBJECT	%VO_2max at 4mmol/l [L]	%VO_2max at 4mmol/l [L] converted with Hct.	Difference (%VO_2max)
AC	82.23	88.60	6.32
ZY	68.04	74.76	6.72
PP	65.52	76.48	10.96
TP	74.37	85.55	11.18
DD	73.0	81.0	8.00
HG	73.50	80.70	7.20
DS	69.48	78.73	9.25
OC	70.00	77.50	7.50
D	60.62	72.01	11.39
CO	73.24	82.80	12.11
RD	71.25	82.00	10.55
MEAN	71.03	80.24	9.20
SD	5.51	5.00	2.12

t = 14,40 and p < 0,001

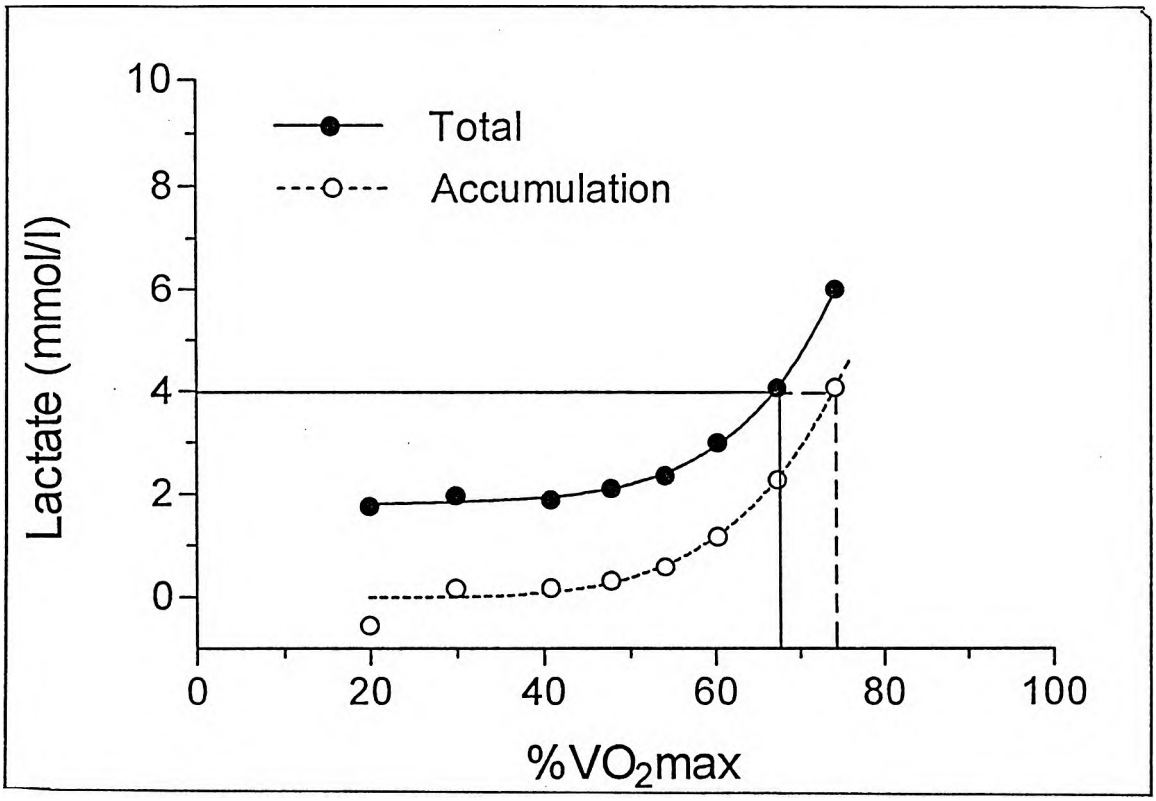

Figure 9: The exponential fit - a comparison of $\%VO_2max$ at a [lactate] of 4mmol/l, before and after converting to accumulated lactate and adjusting for body fluid shifts using Hct values in subject ZY (Test 3).

Table 10: Comparison of the $\%VO_2max$ at [lactate] of 4mmol/l before and after converting to accumulated lactate and adjusting for body fluid shifts with Hct and Hb, when using the exponential model.

SUBJECT	$\%VO_2max$ at 4mmol/l [L]	$\%VO_2max$ at 4mmol/l [L] converted with Hct & Hb	Difference ($\%VO_2max$)
AC	82.28	90.55	8.27
ZY	68.04	73.75	5.71
PP	65.52	78.75	13.23
TP	74.37	85.29	10.92
DD	73.0	80.75	7.75
DS	69.48	77.50	8.02
HG	73.50	80.60	7.01
MEAN	72.31	81.03	8.714
SD	5.44	5.47	2.53

$t = 9,07$ and $p < 0,001$

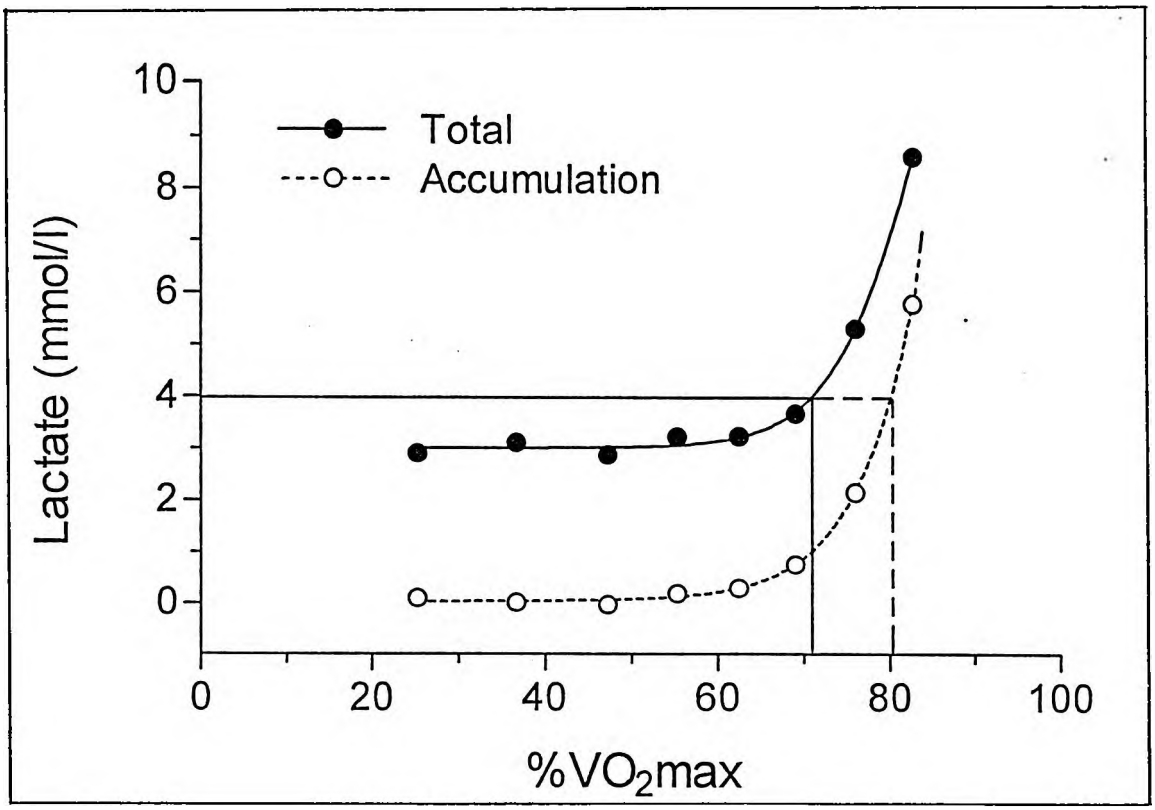

Figure 10: The exponential fit - a comparison of $\%VO_2max$ at a [lactate] of 4mmol/l, before and after converting to accumulated lactate and adjusting for body fluid shifts using Hct and Hb values in subject DS (Test 2).

4.4.3. Comparison of adjusting for body fluid shifts using Hct alone and Hct in combination with Hb.

When comparing $\%VO_2max$ at a lactate concentration of 4mmol/l for data adjusted with Hct or Hct and Hb, the differences using Hct alone appear to be slightly higher than those using Hct and Hb (when observing mean values, Tables 9 and 10). When comparing individual differences, there were varied results and the difference was not statistically significant (Table 11). The appearance of the curves are also similar (figure 11). Once again, adjusting for body fluid shift using Hct only does provide acceptable results.

Table 11: Difference in $\%VO_2max$ at [lactate] of 4mmol/l when adjusting for body fluid shifts using Hct alone and Hct in combination with Hb.

SUBJECT	$\%VO_2max$ at 4mmol/l [L] converted with Hct	$\%VO_2max$ at 4mmol/l [L] converted with Hct & Hb	Difference ($\%VO_2max$)
AC	6.32	8.27	-1.95
ZY	6.72	5.71	1.01
PP	10.96	13.23	-2.27
TP	11.18	10.92	0.26
DD	8.00	7.75	0.25
HG	7.20	7.01	0.19
DS	9.25	8.02	1.23
MEAN	8.52	8.71	0.18
SD	1.99	2.53	1.38

t = 0.35 and there is no significant difference.

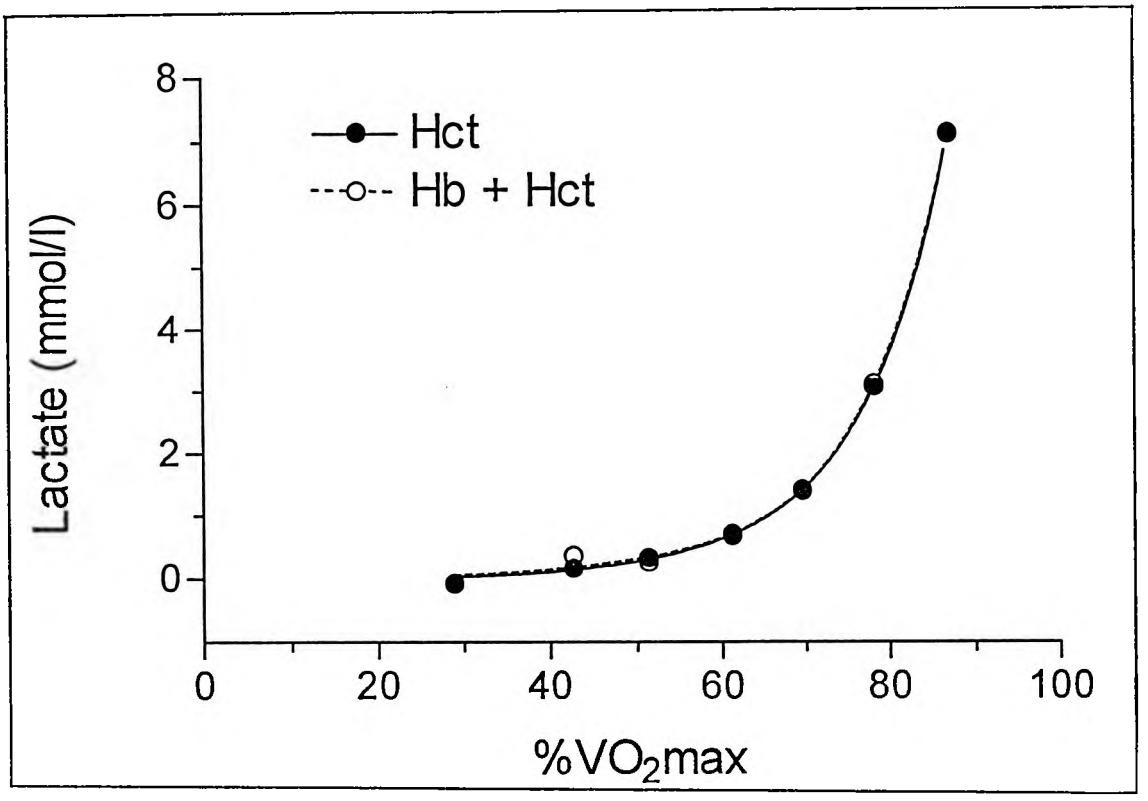

Figure 11: The exponential fit - a comparison of curves adjusted for body fluid shifts using Hct alone and adjusted using a combination of Hct and Hb in subject HG.

CHAPTER 5. DISCUSSION

5.1. **The change in plasma volume during graded exercise.**

During this study, the venous lactate concentrations increased three to six fold during heavy exercise, over the resting values, and the PV decreassed between 3,23 and 18,6%. Even at work rates requiring close to 100% of VO_2max, PV has been reported to rarely decrease by more than 15-18%, provided there is little previous dehydration (26). Sjogaard et al. (70) found that the intracellular lactate concentration was twice that of the extracellular lactate concentration after intense exercise. The lactate concentration gradient therefore favoured a flux of water into the intracellular space, however interstitial water volumes doubled while intracellular water volumes increased only slightly. Osmotic forces due to lactate gradients during short-term, non-steady state activity, cannot therefore be the only factor of importance. They also observed that with exhaustive dynamic exercise, the interstitial water doubled in the active muscle while no changes were observed in the inactive muscles (70).

The response of the intravascular volume to running exercise varies considerably among individuals (26), as was seen in this study. The largest changes were generally seen during the first two or three workloads. Factors which could limit further

haemoconcentration during running are (i) the venous pressure in the legs is reduced due to the action of the muscle pump, (ii) there is movement of water into the intravascular space from inactive tissues as a result of the rise in plasma osmolality during exercise, and (iii) the accumulation of fluid in the active muscle is countered by rising tissue pressure and plasma tonicity, and by changes in the colloid osmotic pressure of plasma and interstitial fluid (26).

On the other hand, an important factor causing an increased filtration of water from the capillary bed into the interstitial space may be an enlarged functional capillary surface area, due to dilation of terminal arterioles and an increased mean capillary pressure. Both capillary hydrostatic pressure and intracellular osmolality contribute to the outward flux of water in the capillaries of the contracting muscles (34,70). If water released from the breakdown of glycogen has an influence on the changes in plasma volume, differing glycogen levels in the active muscles could also explain the variability of the changes in plasma volume.

5.2. Reproducibility of the lactate curve response.

When the lactate curve response was repeated on three different occasions, calculation of accumulated lactate concentrations, corrected for body fluid shifts on each occasion, resulted in a

highly reproducible lactate curve response.

A zero lactate accumulation concentration during the lower exercise intensities indicated that the production and removal of lactate were in balance. Lactate turnover and oxidation rates increase markedly from rest to moderate exercise with only a moderate increase in circulating lactate concentration (25,48,85). During graded exercise, lactate concentration usually increases only slightly above resting values during lower work loads. We found that during lower workloads, the lactate concentration dipped below initial lactate concentrations for some of the subjects, indicating the rate of disappearance (Rd) of lactate was greater than Ra (the rate of lactate appearance. Lactate concentrations increase sharply usually between 50-80% of VO_2max (46,76,81), which was also the case in this study.

5.3. Calculation of body fluid shifts.

Haematocrit values were used independently as this could be a simple test for routine analyses, ie. an acceptable approximation but accurate only when red cell volume is constant. When calculating body fluid shifts, the inclusion of haemoglobin with haematocrit values in the equation provides a more accurate assessment because it takes changes in red blood cell volume into account (20,37,84,85). Human red blood cells can increase, decrease or remain constant in volume during physical stress

depending on the interactions of plasma osmolality and blood pH (84). With maximal exercise, there are very large increments in plasma osmolality, but this influence on the erythrocyte can be neutralised or overcompensated by significant acid-base disturbances (84).

Under the conditions of our study, we found no significant differences in the percent change in plasma volume and the lactate curve response corrected for body fluid shifts when using the equation which included haemoglobin compared to the equation which relied on haematocrit alone. While changes in plasma volume are calculate with greater accuracy using both Hct and Hb, an acceptable result using haematocrit alone would provide a simple test for routine analyses and would be faster and less expensive than using both Hct and Hb. It would also be convenient for use during field trials. It seems, therefore, that the measurement of haematocrit alone constitutes a simple and easy method of correcting for body fluid shifts, and that it would appear to be unnecessary to take the additional precaution of measuring haemoglobin concentration.

5.4. The influence of body fluid shifts on the lactate curve response.

In correcting for the effect of body fluid shifts on lactate concentration during a graded exercise protocol, we needed to

express lactate levels as a concentration of accumulated lactate. When we compared the lactate curves constructed using total lactate concentrations, uncorrected for body fluid shifts, to lactate curves constructed using accumulated lactate concentrations corrected for body fluid shifts, the difference in the lactate threshold (lactate turnpoint) was physiologically marginal even though statistically significant when we excluded the subject with Thallassaemia.

On the other hand, when we fitted exponential curves to the two sets of data, the 4mmol/l thresholds were markedly different. Fitting an exponential function to accumulated lactate concentrations, corrected for body fluid shifts, resulted in a significant alteration in the properties of the curve when compared to the same type of curve fitted to absolute (total) lactate concentrations, not corrected for body fluid shifts.

We conclude that the interpretation of any lactate curve response that neglects to correct for body fluid shifts could result in a significant error.

5.5 Conclusion

The purpose of this study was to determine whether body fluid shifts had any impact on the pattern of lactate accumulation. The rate and extent of plasma volume shifts and lactate

accumulation during physical stress are related to the type and intensity of that stress. When using the classic lactate threshold model, the differences were slight, and although statistically significant could not be considered physiologically significant. The exponential model, on the other hand, showed significant changes in the position of lactate accumulation curve. These changes were not only statistically significant but could also be considered physiologically relevant.

In conclusion, it appears that expressing the lactate curve as an accumulation curve rather than in absolute terms significantly alters the construction of the lactate curve during the continuous, graded exercise used in this study, when using the exponential model.

6. REFERENCES

1. ACEVEDO, E.O. and A.H. GOLDFARB. Increased training intensity effects on plasma lactate, ventilatory threshold and endurance. *Med.Sci.Sports Exerc. 21(5): 563-568, 1989.*

2. BALDWIN, K.M., P.J. CAMPBELL and D.A. COOKE. Glycogen, lactate and alanine changes in muscle fibre types during graded exercise. *J.Appl.Physiol. 43: 288-291, 1977.*

3. BEAVER, W.L., K. WASSERMAN and B.J. WHIPP. Improved detection of lactate threshold using a log-log transformation. *J.Appl.Physiol. 59(6): 1936-1940, 1985.*

4. BEAVER, W.L., K. WASSERMAN and B.J. WHIPP. Blood lactate concentration in exercise. *J.Appl.Physiol. 64(3): 1290-1291, 1988 (Letters to the Editor).*

5. BISHOP, P. and M. MARTINO. Blood lactate measurement in recovery as as adjunct to training. *Sports Med. 16(1): 5-13, 1993.*

6. BOGDANIS, G.C., M.E. NEVILL, L.H. BOOBIS, H.K.A. LAKOMY and A.M.NEVILL. Recovery of power output and muscle metabolites following 30 secs of maximal sprint cycling in man. *J.Physiol. 482(2): 467-480, 1995.*

7. BRANDON, L.J. Physiological factors associated with middle distance running performance. *Sports Med. 19(4): 268-277, 1995.*

8. BROOKS, G.A. Current concepts in lactate exchange. *Med. Sci.Sports Exerc. 23(8): 895-906, 1991.*

9. BROOKS, G.A. Anaerobic threshold: Review of the concept and directions for further research. *Med.Sci.Sports Exerc. 17(1): 22-31, 1985.*

10. BROOKS, G.A. The lactate shuttle during exercise and recovery. *Med.Sci.Sports Exerc. 18(3): 360-368, 1986.*

11. BRUBAKER, P.H., M.J. BERRY, S.C. BROZENA, D.L. MORLEY, J.D. WALTER, A.M. PAOLONE and A.A. BOVE. Relationship of lactate and ventilatory thresholds in cardiac transplant patients. *Med.Sci.Sports Exerc. 25(2): 191-196, 1993.*

12.BULBULIAN, R., A.R. WILCOX and B.A. DARABOS. Anaerobic contribution to distance running performance of trained cross-country athletes. *Med.Sci.Sports Exerc. 18(1); 107-113, 1986.*

13. CAMPBELL, M.E., R.L.HUGHSON and H.J. GREEN. Continuous increase in blood lactate concentration during different ramp exercise protocols. *J.Appl.Physiol. 66(3): 1104-1107, 1989.*

14.CHIRTEL, S.J., R.W. BARBEE and W.N. STAINSBY. Net O_2, CO_2, lactate and acid exchange by muscle during progressive working contractions. *J.Appl.Physiol. 56(1): 161-165, 1984.*

15.COEN, B., L. SCHWARZ, A. URHAUSEN and W. KINDERMANN. Control of training in middle- and long-distance running by means of the individual anaerobic threshold. *Int.J.Sports Med. 12(6): 519-524, 1991.*

16.COGGAN, A.R. Plasma glucose metabolism during exercise in humans. *Sports Med. 11(2): 102-124, 1991.*

17. COLLINS, M.A., K.J. CURETON, D.W. HILL and C.A. RAY. Relation of plasma volume change to intensity of weight training. *Med.Sci.Sports Exerc. 21(2): 178-185, 1989.*

18. CONNET, R.J., T.E.J. GAYESKI and C.R. HONIG. Lactate accumulation in fully aerobic, working dog gracilis muscle. *Am.J.Physiol. 246(1): H120-H128, 1984.*

19. COSTILL, D.L. and W.J. FINK. Plasma volume changes following exercise and thermal dehydration. *J.Appl.Physiol. 37(4): 521-525, 1974.*

20. COSTILL, D.L., L. BRANAM, D. EDDY and W. FINK. Alterations in red cell volume following exercise and dehydration. *J.Appl.Physiol. 37(6): 912-916, 1974.*

21. COSTILL, D.L. and B. SALTIN. Changes in the ratio of venous to body haematocrit following dehydration. *J.Appl.Physiol. 36(5): 608-610, 1974.*

22. COSTILL, D.L. and M. HARGREAVES. Carbohydrate nutrition and fatigue. *Sports Med. 13(2): 86-89, 1992.*

23. DAVIS, J.A. Anaerobic threshold: Review of the concept and directions for further research. *Med.Sci.Sports Exerc. 17(1): 6-18, 1985.*

24. DILL, D.B. and D.L. COSTILL. Calculation of percentage changes in volumes of blood, plasma and red cells in dehydration. *J.Appl.Physiol. 37(2): 247-248, 1974.*

25. DONOVAN, C.M. and G.A.BROOKS. Endurance training affects lactate clearance, not lactate production. *Am.J.Physiol. 244 (7): E88-E92, 1983.*

26. EDWARDS, R.J. and M.H. HARRISON. Intravascular volume and protein responses to running exercise. *Med.Sci.Sports Exerc. 16(3): 247-255, 1984.*

27. ELDRIDGE, F.L. Relationship between turnover and blood concentration of lactate in exercising dogs. *J.Appl.Physiol. 39: 231-234, 1975.*

28. FARREL, P.A., J.H. WILMORE, E.F. COYLE, J.E. BILLING and D.L. COSTILL. Plasma lactate accumulation and distance running performance. *Med.Sci.Sports Exerc. 11(4): 338-344, 1979.*

29. FLEG, J.L., S.P.TZANKOFF and E.G. LAKATTA. Age-related augmentation of plasma catecholamines during dynamic exercise in healthy males. *J.Appl.Physiol. 59(4): 1033-1039, 1985.*

30. GAESSER, G.A. and D.C. POOLE. Lactate and ventilatory thresholds: disparity in time course of adaptions to training. *J.Appl.Physiol. 61(3): 999-1004, 1986.*

31. GOLLNICK, P.D., W.M. BAYLY and D.R. HODGSON. Exercise intensity, training, diet and lactate concentration in muscle and blood. *Med.Sci.Sports Exerc. 18(3): 334-340, 1986.*

32. GREEN, H.J., R.L. HUGHSON, G.W. ORR and D.A. RANNEY. Anaerobic threshold, blood lactate and muscle metabolites in progressive exercise. *J.Appl.Physiol. 54(4): 1032-1038, 1983.*

33. GREEN, S. and B. DAWSON. Measurement of anaerobic capacities in humans. *Sports Med. 15(5): 312-327, 1993.*

34. GREENLEAF, J.E.,V.A. CONVERTINO, R.W. STREMEL, E.M. BERNAUER, W.C. ADAMS, S.R. VIGNAU and P.J. BROCK. Plasma [Na+], [Ca^{2+}] and volume shifts and thermoregulation during exercise in man. *J.Appl.Physiol. 43(6): 1026-1032, 1977.*

35. GUYTON, Human physiology and mechanisms of disease. 5th ed. Chapter 45: Metabolism of Carbohydrates and Formation of Adenosine Triphosphate, pgs. 514-519; Chapter 57: Sports Physiology, pgs 640-647. Philadelphia: H.B. Saunders. 1992.

36. HAGBERG,J.A., E.F.COYLE, J.E. CARROLL, J.M. MILLER, W.H. MARTIN and M.H. BROOKE. Exercise hyperventilation in patients with McArdle's disease. *J.Appl.Physiol. 52(4): 991-994, 1982.*

37. HARRISON, M.H., R.J. EDWARDS and D.R. LEITCH. Effect of exercise and thermal stress on plasma volume. *J.Appl.Physiol. 39(6): 925-931, 1975.*

38. HEIGENHAUSER, G.J.F., J.R. SUTTON and N.L. JONES. Effect of glycogen depletion on the ventilatory response to exercise. *J.Appl.Physiol. 54(2): 470-474, 1983.*

39. HEITKAMP, H-Ch., M. HOLDT and K. SCHEIB. The reproducibility of the 4mmol/l lactate threshold in trained and untrained women. *Int.J.Sports Med. 12(4): 363-368, 1991.*

40. HERMANSEN, L. and O. VAAGE. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. *Am.J.Physiol. 233(5): E422-E429, 1977.*

41. HOGAN, M.C., L.B. GLADDEN, S.S. KURDAK and D.C. POOLE. Increased [lactate] in working dog muscle reduces tension development independent of pH. *Med.Sci.Sports Exerc. 277(3): 371-377, 1995.*

42. HOGAN, M.C., R.H. COX and H.G. WELCH. Lactate accumulation during incremental exercise with varied inspired oxygen fractions. *J.Appl.Physiol. 55(4): 1134-1140, 1983.*

43. HOGAN, M.C. and H.G. WELCH. Effect of varied lactate levels on bicycle ergometer performance. *J.Appl.Physiol. 57(2): 507-513, 1984.*

44. HUGHES, E.F., S.C. TURNER and G.A. BROOKS. Effects of glycogen depletion and pedalling speed on the anaerobic threshold. *J.Appl.Physiol. 52(5): 1598-1607, 1982.*

45. HUGHSON, R.L., H.J. GREEN and M.T. SHARRATT. Gas exchange, blood lactate and plasma catecholamines during incremental exercise in hypoxia and normoxia. *J.Appl.Physiol. 79(4): 1134-1141, 1995.*

46. HUGHSON, R.L., K.H. WEISIGER and G.D. SWANSON. Blood lactate concentration increases as a continuous function in progressive exercise. *J.Appl.Physiol. 62(5): 1975-1981, 1987.*

47. IDSTROM J-P., V.HARIHARA SUBRAMANIAN, B. CHANCE, T. SCHERSTEN and A-C. BYLUND-FELLENIUS. Oxygen dependence of energy metabolism in contracting and recovering rat skeletal muscle. *Am.J.Physiol. 248(17): H40-H48, 1985.*

48. ISSEKUTZ, B.JR. Effect of B-adrenergic blockade on lactate turnover in exercising dogs. *J.Appl.Physiol. 57(6): 1754-1759, 1984.*

49. KANSTRUP, I-L. and B. EKBLOM. Blood volume and haemoglobin concentration as determinants of maximal aerobic power. *Med.Sci. Sports Exerc. 16(3): 256-262, 1984.*

50. KATZ, J. The application of isotopes to the study of lactate metabolism. *Med.Sci.Sports Exerc. 18(3): 353-359, 1986.*

51. KATZ, A. and K. SAHLIN. Regulation of lactic acid production during exercise. *J.Appl.Physiol. 65(2): 509-518, 1988.*

52. KIRWIN, J.P., D.L. COSTILL, J.B. MITCHELL. Carbohydrate balance in competitive runners during successive days of intense training. *J.Appl.Physiol. 65(6): 2601-2606, 1988.*

53. LEHMAN, S.L. Measurement of lactate production by tracer techniques. *Med.Sci.Sports Exerc. 23(8): 935-938, 1991.*

54. LOAT, C. and E.C. RHODES. Relationship between lactate and ventilatory thresholds during prolonged exercise. *Sports Med. 15(2): 104-113, 1993.*

55. LUNDVALL, J., S. MELLANDER, H. WESTLING and T. WHITE. Fluid transfer between blood and tissues during exercise. *Acta.Physiol.Scand. 85: 258-269, 1972.*

56. MAZZEO, R.S. and P. MARSHALL. Influence of plasma catecholamines on the lactate threshold during graded exercise. *J.Appl.Physiol. 67(4): 1319-1322, 1989.*

57. MCARDLE, W.D., F.I. KATCH and V.L. KATCH. Exercise Physiology, energy, nutrition and human performance. 3rd Ed. Chapter 6: Energy Transfer in the Body, pgs. 108-116; Chapter 7: Energy Transfer in Exercise, pgs. 125-141; Chapter 11: Individual differences and measurement of Energy Capacities, pgs. 205-210; Chapter 14: Dynamics of Pulmonary Ventilation, pgs. 278-281 and 285-289. Philadelphia: Lea and Febiger, 1991.

58. MCLELLAN, T.M. and G.C. GASS. Metabolic and cardiorespiratory responses relative to the anaerobic threshold. *Med.Sci.Sports Exerc. 21(2): 191-198, 1989.*

59. MCLELLAN, T.M., KS.Y. CHEUNG and I. JACOBS. Incremental test protocol, recovery mode and the individual anaerobic threshold. *Int.J.Sports Med. 12(2): 190-195, 1991.*

60. MYERS, J., D. WALSH, N. BUCHANAN, P. MCAULEY, E. BOWES and V. FROELICHER. Increase in blood lactate during ramp exercise: comparison of continuous and threshold models. *Med.Sci.Sports Exerc. 26(11): 1413-1419, 1994.*

61. NOAKES, T.M. Lore of running. 2nd Edition, Chapter 2: Physiology and biochemistry of running, pgs.67-74. Oxford, 1986.

62.ORR, G.W., R.L. HUGHSON, H.J. GREEN and G.W. BENNETT. A computer linear regression model for determination of ventilatory anaerobic threshold. *J. Appl. Physiol. 52:1349-1352, 1982.*

63. POOLE, D.C. and G.A. GAESSER. Response of the ventilatory and lactate thresholds to continuous and interval training. *J.Appl.Physiol. 58(4): 1115-1121, 1985.*

64. RICHTER, E., N. RUDERMAN, H. GARVAS, E. BULUR and H. GALBO. Muscle glycogenolysis during exercise: dual control by epinephrine and contractions. *Am.J.Physiol. 242 (5): E25-E32, 1982.*

65. ROBERTS, D. and D.J. SMITH. Biochemical aspects of peripheral muscle fatigue. *Sports Med. 7: 125-138, 1989.*

66. ROTH, D.A. The sarcolemmal lactate transporter: transmembrane determinants of lactate flux. *Med.Sci.Sports Exerc. 23(8): 925-934, 1991.*

67. SCHNEIDER, D.A. and J. POLLACK. Ventilatory threshold and maximal oxygen uptake during cycling and running in female triathletes. *Int.J.Sports Med. 12(4): 379-383, 1991.*

68. SEGAL, S.S. and G.A. BROOKS. Effects of glycogen depletion and workload on post exercise O_2 consumption and blood lactate. *J.Appl.Physiol. 47: 514-521, 1979.*

69. SIMON, J., J.L. YOUNG, D.K. BLOOD, K.R. SEGEL, R.B. CASE and B. GUTIN. Plasma lactate and ventilation thresholds in trained and untrained cyclists. *J.Appl.Physiol. 60(3): 777-782, 1986.*

70. SJOGAARD, G. and B. SALTIN. Extra- and intracellular water spaces in muscles of man at rest and with dynamic exercise. *Am.J.Physiol. 243(12): R271-R280, 1982.*

71. STAINSBY W.N. Biochemical and physiological bases for lactate production. *Med.Sci.Sports Exerc. 18(3): 341-343, 1986.*

72. STAINSBY W.N., W.F. BRECHUE and D.M. O'DROBINAK. Regulation of muscle lactate production. *Med.Sci.Sports Exerc. 23(8): 907-911, 1991.*

73. STAINSBY W.N., C. SUMNERS and P.D. EITZMAN. Effects of adrenergic agonists and antagonists on muscle O_2 uptake and lactate metabolism. *J.Appl.Physiol. 62(5): 1845-1851, 1987.*

74. STAINSBY W.N., C. SUMNERS and M. ANDREW. Plasma catecholamines and their effect on blood lactate and muscle lactate output. *J.Appl.Physiol. 57(2): 321-325, 1984.*

75. STAINSBY W.N., C. SUMNERS and P.D. EITZMAN. Effects of catecholamines on lactic acid output during progressive working contractions. *J.Appl.Physiol. 59(6): 1809-1814, 1985.*

76. STANLEY, W.C., E.W. GERTZ, J.A. WISNESKI, D.L. MORRIS, R.A. NEESE and G.A.BROOKS. Systemic lactate kinetics during graded exercise in man. *Am.J.Physiol. 249 (12): E595-E602, 1985.*

77. STANLEY, W.C. Myocardial lactate metabolism during exercise. *Med.Sci.Sports Exerc. 23(8): 920-924, 1991.*

78. TANAKA, H., S. FUKUMOTO, Y. OSAKA, H. YAMAGUCHI and H. MIYAMOTO. Distinctive effects of three different modes of exercise on oxygen uptake, heart rate and blood lactate and pyruvate. *Int.J.Sports Med. 12(5): 433-438, 1991.*

79. TANAKA, K., Y. MATSUURA, A.MATSUZAKA, K.HIRAKOBA, S. KUMAGAI, S.O.SUN and K. ASANO. A longitudinal assessment of anaerobic threshold and distance running performance. *Med.Sci. Sports Exerc. 16(3): 278-282, 1984.*

80. TANAKA, K. and Y. MATSUURA. Marathon performance, anaerobic threshold and onset of blood lactate accumulation. *J.Appl.Physiol. 57(3): 640-643, 1984.*

81. TURNER, M.J., E.T. HOWLEY, H. TANAKA, M. ASHRAF, D.R. BASSETT,JR. and D.J.KEEFER. Effect of graded epinephrine infusion on blood lactate response to exercise. *J.Appl. Physiol. 79(4): 1206-1211, 1995.*

82. VAN BEAUMONT, W., J.E. GREENLEAF and L. JUHOS. Disproportional changes in haematocrit, plasma volume and proteins during exercise and bed rest. *J.Appl.Physiol. 33(1): 55-61, 1972.*

83. VAN BEAUMONT, W., J.C. STRAND, J.S. PETROFSKY, S.G. HIPSKIND and J.E. GREENLEAF. Changes in total plasma content of electrolytes and proteins with maximal exercise. *J.Appl. Physiol. 34(1): 102-106, 1973.*

84. VAN BEAUMONT, W., S. UNDERKOFLER and S. VAN BEAUMONT. Erythrocyte volume, plasma volume and acid-base changes in exercise and heat dehydration. *J.Appl.Physiol. 50(6): 1255-1262, 1981.*

85. VAN BEAUMONT, W. Red cell volume with changes in plasma osmolarity during maximal exercise. *J.Appl.Physiol. 35(1): 47-50, 1973.*

86. WASSERMAN, D.H., D.B. LACY, D.R. GREEN, P.E. WILLIAMS and A.D. CHERRINGTON. Dynamics of hepatic lactate and glucose balances during prolonged exercise and recovery in the dog. *J.Appl.Physiol. 63(6): 2411-2417, 1987.*

87. WASSERMAN, D.H., C.C. CONNOLLY and M.J. PAGLIASSOTTI. Regulation of hepatic lactate balance during exercise. *Med.Sci.Sports Exerc. 23(8): 912-919, 1991.*

88. WASSERMAN, K., B.J. WHIPP, S.N. ROYAL and W.L. BEAVER. Anaerobic threshold and respiratory gas exchange during exercise.

J.Appl.Physiol. 35: 236-243, 1973.

89. WASSERMAN, K., W.L.BEAVER and B.J. WHIPP. Mechanisms and patterns of blood lactate increase during exercise in man. *Med.Sci.Sports Exerc. 18(3): 344-352, 1986.*

90. WASSERMAN, K., W.L. BEAVER, J.A. DAVIS, J-Z. PU, D. HEBER and B.J. WHIPP. Lactate, pyruvate and lactate-to-pyruvate ratio during exercise and recovery. *J.Appl.Physiol. 59(3): 935-940, 1985.*

91. WINDER, W.W. Control of hepatic glucose production during exercise. *Med.Sci.Sports Exerc. 17(1): 2-5, 1985.*

92. YEH M.P., R.M. GARDNER, T.D. ADAMS, F.G. YANOWITZ and R.O. CRAPO. "Anaerobic threshold": Problems of determination and validation. *J.Appl.Physiol. 55(4): 1178-1186, 1983.*

APPENDIX

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 (Registry)

CLEARANCE CERTIFICATE · PROTOCOL NUMBER M931007

PROJECT	Kinetics of lactate production
INVESTIGATORS	Ms M A Castleman
DEPARTMENT	Physiology
DATE CONSIDERED	931029

DECISION OF THE COMMITTEE

Approved with the attached condition

DATE 931102 CHAIRMAN .....
(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.,

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE ..... SIGNATURE .....