THE EFFECTS OF ENDURANCE TRAINING ON LACTATE PRODUCTION AND REMOVAL DURING PROGRESSIVE EXERCISE IN MAN

by

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To my wife Priscilla, whose steadfast love

and support are a constant reminder

of the special person she is.

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ABSTRACT

It is a well-documented finding that blood lactate concentrations at any given absolute or relative (% of maximum oxygen uptake; % VO_2max) workload, are lower following endurance training. The search for the mechanisms responsible for lower blood lactate concentrations after training, however, has led to conflicting results, particularly when the possible causes of this finding have been investigated in humans. In this study, three questions related to the effects of endurance training on lactate metabolism were investigated. Firstly, it was determined whether the reduced blood lactate concentrations [La] during submaximal exercise, in humans, after endurance training is the result of a decreased rate of lactate appearance (Ra) or an increased rate of lactate metabolic clearance (MCR). Interrelationships among blood [La], lactate Ra and MCR were investigated in 8 untrained men during progressive (60 W + 40 W every 6 min) exercise to exhaustion, before and after a 9 week endurance training programme. While the subjects exercised at increasing work rates, L-(U-^{14}C) lactate was infused intravenously into the one forearm and "arterialized" venous blood was sampled every 3 min from the other forearm. Radioisotope dilution measurements revealed that the slower rise in blood [La] with increasing VO_2 following training, was due to the combined effect of a reduced lactate Ra and an increased MCR. After training decreases in lactate Ra were most pronounced at the lower work rates (VO_2's < 2.27 l.min^{-1}, < 60% VO_2max; $p < 0.01$). At work rates closer to exhaustion, lactate Ra values before and after training became similar. Both before and after training, subjects terminated exercise when their blood lactate Ra rose to 215 ± 28 and 244 ± 12 umol.min^{-1}.kg^{-1} respectively. In contrast, submaximal (< 75% VO_2max), and peak lactate MCR values were higher after vs. before training (40 ± 3 vs. 31 ± 4 ml.min^{-}

$^{1}.kg^{-1}$, $p < 0.01$). Thus, the attenuated blood lactate accumulation after training was due to a diminished lactate Ra at low absolute and relative work rates, and an elevated MCR at higher absolute and all relative work rates during exercise.

Secondly, this study investigated the effects of endurance training on the relative contributions of oxidation (lac R_{ox}) and gluconeogenesis (lac R_{glu}) to lactate removal during exercise. Blood ^{14}C-lactate and ^{14}C-glucose measurements revealed that training decreased the percentage of glucose labeled by the transfer of ^{14}C from lactate. After training, estimated rates of glucose synthesis from lactate were reduced from 66 ± 6 to 54 ± 5 umol.min^{-1}. kg^{-1} at a VO_2 of 1.06 l.min^{-1}, and from 110 ± 15 to 57 ± 6 umol.min^{-1}.kg^{-1} at a VO_2 of 2.27 l.min^{-1} ($p< 0.01$). With less blood lactate accumulation, $^{14}CO_2$ expiration from the oxidation of ^{14}C-lactate, and ^{14}C-glucose from lactate was also lower at all VO_2's ($p< 0.01$). However, at equivalent blood [La]'s, training increased the estimated rates of oxidation (lac R_{ox}). At 2 mmol.l^{-1}, lac R_{ox} was increased from 9 ± 1 to 20 ± 3 umol.min^{-1}.kg^{-1} ($p< 0.01$) and, at 6 mmol.l^{-1}, lac R_{ox} was raised from 38 ± 4 to 72 ± 10 umol.min^{-1}.kg^{-1} ($p< 0.01$). In contrast, the rates of removal of 2 and 6 mmol.l^{-1} blood [La]'s before and after training were similar. It was therefore concluded that, at equivalent metabolic rates after training, lactate removal via gluconeogenesis and oxidation is reduced, and that at equivalent blood [La]'s after training only the removal of lactate via oxidation is significantly improved.

Thirdly, continuous exponential rises in blood [La] with increasing VO_2 have recently received attention as being more indicative of the response of lactate to progressively high work rates, than the traditional "threshold model" proposed by Wasserman et al.

(1973). Data from this investigation (a) support the exponential model of lactate accumulation with increasing work rate, and (b) show the effects of training and exercise ramp on the blood lactate response to incremental work. Specifically, two exercise protocols were employed on separate days before training and on separate days after training. In the first protocol, arterialized venous blood was sampled each minute during 20W. min^{-1} non-steady state incremental exercise to exhaustion. The second protocol required progressive (60 W + 40 W every 6 min) exercise to exhaustion. Metabolic and blood [La^-] responses to the exercise ramps were fitted with linear and non-linear [Y = A. exp.(B. X.) + C] regression equations. The l. min^{-1} VO_2's at dLa/dVO_2 "slopes" of 1, 2, and 3 were higher in both exercise ramps after training ($p < 0.05$), thereby reflecting the markedly attenuated rates of blood [La-] accumulation seen during exercise after training. Blood [La^-] increased exponentially during exercise before and after training. Accelerations of minute ventilation volume (V_I) expressed as dV_I/dVO_2 at the 1, 2, and 3 "lactate slopes", showed that training dissociated the continuous increase in ventilation volume from the exponential rise in blood [La^-].

In summary, the diminished blood lactate accumulation during submaximal exercise after training, was due to reduced rates of lactate appearance at low work rates, and improved rates of lactate clearance at higher work rates. The removal of the lactate formed during exercise, via oxidation and gluconeogenesis, was reduced at equivalent metabolic rates after training. However, lactate removal via oxidation was increased at equivalent blood [La]'s after training. The "anaerobic threshold" hypothesis was rejected, since blood [La]'s increased exponentially during both fast and slow ramp exercise tests before and after training, and training dissociated the continuous increase in minute ventilation from the exponential rise in blood [La].

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CHAPTER ONE

Introduction to the Problem

The concentration of lactate increases or declines in different tissues such as muscle and blood at rest and during exercise because the rates of lactate production and removal differ. Constant tissue and blood lactate concentrations indicate that the rates of lactate production and lactate removal are equal. Tissues that release lactate into the plasma include Type 2b skeletal muscle fibres and erythrocytes. Tissues that oxidize lactate produced at rest or during exercise include Type 1 skeletal muscle fibres, cardiac muscle, the kidney cortex and the liver. The liver exhibits a dual role in lactate metabolism. After prolonged fasting lactate uptake dominates and immediately after feeding lactate output is evident. Plasma lactate concentrations are traditionally used as an indicator of the extent to which non-oxidative pathways (glycolytic) are activated during exercise to generate energy for phosphorylation of ADP. The use of plasma lactate concentrations to reflect lactate production and removal rates is not feasible. The rates of lactate production or oxidation in the intact human can only be assessed with the benefit of lactate tracer methodology.

Statement of the Problem

Numerous studies have demonstrated that during incremental exercise to exhaustion, oxygen consumption increases linearly, but blood lactate concentrations do not change markedly until an exercise intensity of approximately 60% of the maximal oxygen uptake (VO_2max) in untrained, and 80 to 90% VO_2max in trained individuals has been reached. The blood lactate concentration then begins to increase nonlinearly. The point at which this sudden increase commences is referred to as the anaerobic threshold, lactate inflection point,

lactate turnpoint, lactate threshold, onset of plasma lactate accumulation (OPLA), and onset of blood lactate accumulation (OBLA). The lactate threshold has been attributed to motor unit recruitment patterns, catecholaminergic stimulation, hypoxia, temperature effects, substrate utilization, decreased lactate removal, metabolic stimulation, and threshold of lactate efflux, as recently reviewed by Walsh and Banister (Walsh, & Banister, 1988). These factors are believed to be the major determinants of the lactate threshold. Recent studies have questioned whether there is in fact a true lactate threshold (Campbell, Hughson, & Green, 1989; Dennis, MacRae, & Noakes, 1989; Dennis, Noakes, & Bosch, 1991; Hughson, Weisiger, & Swanson, 1987; Morton, 1989). Rather, these studies suggest that there is an exponential increase in blood lactate concentration with increasing rates of work. These more recent investigations conflict with the traditional acceptance of a lactate threshold as described by Wasserman et al. (Wasserman, Whipp, Koyal, & Beaver, 1973). As this issue is currently unresolved, the potentially incorrect term, the lactate threshold, will be used in this thesis.

Several studies have also demonstrated that the concentration of blood lactate is reduced at the same absolute exercise intensity after endurance training (Denis, Dormois, & Lacour, 1984; Donovan, & Brooks, 1983; Gaesser, & Poole, 1986). Recent studies have also shown that endurance training alters the lactate threshold so that, after training, an individual must exercise at a higher percentage of the VO_2max in order to reach the same blood lactate concentration as before training (Hurley, et al., 1984; Robinson, & Harmon, 1941). In addition, significant increases in the exercise intensity at the lactate threshold have been shown to occur within the first few weeks of training (Gaesser, et al., 1986). The biochemical mechanisms responsible for this training-induced increase in the lactate threshold and the lower blood lactate concentrations observed in response to training, remain to be clarified.

The historical explanation for the lower blood lactate concentrations following endurance training is that there is a decreased rate of lactate production in skeletal muscles during submaximal exercise (Henriksson, 1977; Holloszy, & Coyle, 1984; Saltin, et al., 1976). This decreased rate of production of lactate is believed to result from an increased rate of free fatty acid oxidation to supply energy for contraction, with a consequent reduction in the rate of glycolysis in the exercising, trained muscle. These data conflict with recent studies of lactate kinetics using tracer methodology in rats and humans. Donovan and Brooks (Donovan, et al., 1983), Mazzeo et al. (Mazzeo, Brooks, Schoeller, & Budinger, 1986), and Stanley et al. (Stanley, et al., 1985; Stanley, Wisneski, Gertz, Neese, & Brooks, 1988), have proposed that the rate of lactate production is not altered by exercise training, but that the lower blood lactate concentrations observed in the trained state are due to an increased rate of lactate clearance from the blood.

Thus it is clear that the effects of exercise training on lactate kinetics, especially in humans, remain to be established.

Scope of the Study

This study attempts to determine, for the first time in humans, whether the reduction in blood lactate concentrations during submaximal exercise after training, results from a reduction in the rate of lactate production or from an increase in the rate of lactate clearance, or both. This question was investigated in 8 untrained men by infusing L-(U-^{14}C)-lactate before and after a 9 week training programme. This study also attempted to determine the metabolic fate of lactate produced during exercise. In addition, the effects of different incremental exercise test protocols on changes in blood lactate concentration were also determined. An additional purpose of this study was to identify the metabolic mechanism(s)

responsible for the increase in exercise intensity that can be achieved after training before lactate begins to accumulate in the blood.

It was therefore hypothesized that the lower blood lactate concentrations observed in response to endurance exercise training, result from a reduction in the rate of production of lactate and an increase in the rate of clearance of lactate from the blood.

Limitations of the Study

In this investigation, L-(U-^{14}C)-lactate was infused venously and blood samples were taken from a contralateral heated ("arterialized") forearm vein (v-v_a mode). Various investigators have compared the method of aortic infusion and venacaval sampling of blood (a-v mode) with the method of venacaval infusion and aortic sampling of blood (v-a mode), particularly since the specific activity of lactate depends on where the tracer is infused or injected, and where blood is sampled (Katz, 1982; Katz, Okajima, Chenoweth, & Dunn, 1981). The a-v mode, as suggested by Katz and associates, is not appropiate for human experimentation. In order to achieve adequate mixing of radioactive lactate with endogenous lactate, previous studies on exercising humans have used the v-a infusion mode (Stanley, et al., 1985; Stanley, et al., 1986; Stanley, et al., 1988). The resting lactate turnover values observed in this study are similar to those reported for the v-a mode, as well as for a previous study which used the v-v mode (Mazzeo, et al., 1986). It has also recently been demonstrated that similar arterialized and deep venous blood ^{14}C-lactate specific activities are achieved using the technique employed in this study (Virkamaki, Puhakainen, Nurjhan, Gerich, & Yke-Jarvinen, 1990).

Lehman and Brooks (Lehman, & Brooks, 1990) recently presented data to show that arterial and venous blood specific activities need to be transformed. They suggest that mixed venous blood represents a more accurate blood sample in lactate tracer studies. We therefore performed calculations of mixed-venous lactate SA (SA_{mv}) from our "arterialized" venous lactate SA as follows:

$$SA_{mv} = SA_{art} - [I / (Q\ [La])]$$

where SA_{mv} is mixed venous SA in $dpm.umol^{-1}$, SA_{art} is arterial SA in $dpm.umol^{-1}$, I is the infusion rate in $dpm.min^{-1}$, Q is the estimated cardiac output in $ml.min^{-1}$ (Saltin, 1969) and [La] is the "arterialized" venous blood lactate concentration in $umol.ml^{-1}$.

The SA_{mv} values gave slightly higher estimates of lactate Ra and Rd compared to the SA_{art} values, but, the differences were not significant (lactate Ra shown in Figure 1.1 A). More to the point, the pattern was the same. At VO_2's of 1.06 $l.min^{-1}$ lactate Ra was reduced from 112±15 to 81±9 $umol.min^{-1}.kg^{-1}$ after training and, at VO_2's of 2.27 $l.min^{-1}$ it was decreased from 252±36 to 136±15 $umol.min^{-1}.kg^{-1}$ (Figure 1.1 B; $p < 0.01$). Therefore, the data presented in this thesis are for the venous infusion and "arterialized" venous sampling mode ($v\text{-}v_a$).

An additional limitation of using L-(U-^{14}C)-lactate as a tracer is its equilibration with pyruvate through the reversible LDH reaction. Unfortunately, the extent to which isotope equilibration between ^{14}C-lactate and pyruvate, via the lactate permease and lactate dehydrogenase equilibria, influences the estimates of lactate turnover and oxidation is difficult to assess. In this study, some pyruvate would also have been labeled by the progressive transfer of ^{14}C from lactate to glucose.

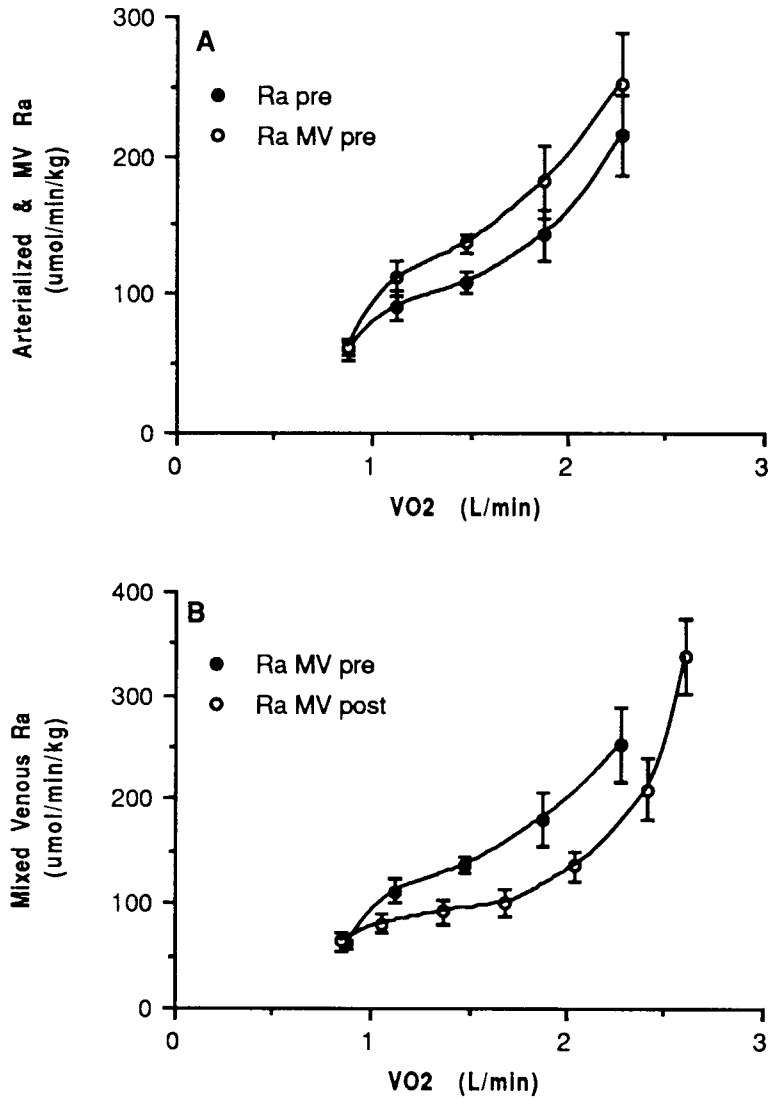


FIGURE 1.1. Comparison between "arterialized" venous (Ra) and estimated mixed venous lactate Ra (Ra MV) before and after training. No significant difference was evident between the two modes before training (panel A). Estimated mixed venous lactate Ra was lower at a VO_2 of 1.06 to 2.27 $l.min^{-1}$ after training (panel B; $p < 0.01$).

The only indication of how glucose turnover might have affected the estimates of lactate turnover, as a consequence of training, is provided by Coggan et al. (Coggan, Kohrt, Spina, Bier, & Holloszy, 1990). They showed that training decreased the turnover of glucose, at a VO_2 of 1.9 $l.min^{-1}$, from 100 to 72 umol C_3 $units.min^{-1}.kg^{-1}$. In this study, the equivalent lactate Ra and Rd values were reduced from around 140 and 110 to 80 $umol.min^{-1}.kg^{-1}$.

An additional limitation of this study is the estimation of the rates of lactate removal via oxidation and gluconeogenesis from expired $^{14}CO_2$. During exercise, some of the carbon dioxide from carbohydrate (CHO) oxidation may be trapped in the HCO_3^- pool, and would therefore not be accounted for in the estimation of lactate removal via oxidation and/or gluconeogenesis. Previous exercise studies, however, have indicated that this is a minor problem because of the rapid turnover of bicarbonate at high CO_2 production rates (Mazzeo, et al., 1986).

CHAPTER TWO

Overview of Lactate Metabolism during Exercise

Production of energy during exercise of high intensity (> 90% of the maximum oxygen consumption; VO_2max) depends largely on the availability of muscle glycogen and blood glucose. Although it is possible to perform light exercise with low levels of these carbohydrates, depletion of these fuels makes it impossible for the skeletal muscles to meet energy requirements and sustain contractile tension needed for work performance (Costill, Gollnick, Jansson, Saltin, & Stein, 1973a). Although fat and protein contribute to the energy pool used during muscular activity, carbohydrate (CHO) is the primary fuel for exercise of high intensity.

It is known that muscle glycogenolysis can be stimulated firstly during muscle contractions via calcium increasing phosphorylase *a* activity via the activation of phosphorylase *b* kinase, and secondly by epinephrine (E) which increases cyclic AMP formation, ultimately leading to the conversion of phosphorylase *b* to phosphorylase *a* (Fischer, Heilmayer, & Haschke, 1971; Soderling, & Park, 1974). This sequence of events begins the two distinct phases of CHO oxidation to CO_2 and H_2O. The first phase, glycolysis, involves a series of reactions that degrades a 6-carbon hexose to two 3-carbon trioses. This series of reactions, known as the Embden-Meyerhof pathway, occurs in the cytosol and results in the production of pyruvate. Pyruvate formation is associated with a stoichiometric reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH. To maintain glycolysis, thereby producing ATP for energy requiring processes and substrate for the tricarboxylic acid cycle (TCA cycle), NADH must be continuously reoxidized to NAD^+. Oxidation of NADH occurs in the mitochondria or through the lactate dehydrogenase reaction. This latter

reaction is of particular importance during conditions of accelerated glycolysis, especially at exercise intensities equal to, or above approximately 50-70% VO_2max. In this reaction, pyruvate is reduced by interacting with NADH to form lactate, thereby permitting the continued oxidation of 3-phosphoglyceraldehyde. Once formed via glycolysis, lactate may be removed via either oxidation or conversion to glucose.

The sites of lactate removal via oxidation are generally considered to be the heart, kidney cortex, and slow and fast-twitch oxidative skeletal muscle fibres. Any lactate which is produced in excess of that which can be oxidized via the Krebs cycle or taken up for glucose/glycogen synthesis during or after muscle activity, could be transported across the sarcolemmal membrane via three possible pathways: diffusion of the undissociated lactic acid (non-ionic diffusion), transport via the lactate (monocarboxylate) carrier (Roth, & Brooks, 1990a; Roth, & Brooks, 1990b), or mediated via the inorganic anion-exchange system (Juel, 1988).

The classic studies by Hill and Lupton (1923) examined the interrelationships between muscular exercise, lactate, and the delivery and utilization of oxygen in both the isolated frog muscle preparation and in man. From their experiments they inferred that during steady-state exercise in one male subject, lactate concentration in the active musculature gradually increased above resting values. They suggested that provided the oxygen supply was adequate, production of lactate was balanced by its oxidative removal as long as the intensity of exercise was kept constant. When their subject was exposed to a higher speed of running however (16 km. h^{-1}), they stated that " the lactic acid is continuously accumulating in the muscles, the maximal oxygen intake ... being inadequate to maintain the recovery at a level high enough to cope with the production of lactic acid". The results of these early investigations led Hill et al. to believe that lactate was essential for muscle

contraction. They considered it to be the signal for muscle contraction, a role which today would be ascribed to Ca^{++}. Once lactate was formed, Hill et al. believed that it was removed either by oxidative metabolism, or by resynthesis to glucose/glycogen. Energy for glucose/glycogen resynthesis was provided by the oxidative removal of some of the formed lactate. Thus, if lactate accumulated, it meant that oxidative metabolism was inadequate to meet the energy requirements of the individual.

A subsequent series of experiments by Hill et al. (1925) demonstrated again that at low intensities of exercise lactate does not accumulate in the blood, whereas at high intensities of exercise lactate accumulates in muscle and blood, due they believed, to the limitations of oxygen supply. Jervell (Jervell, 1928), Margaria et al. (Margaria, Edwards, & Dill, 1933), Bang (Bang, 1936), and Newman et al. (Newman, Dill, Edwards, & Webster, 1937) all provided further evidence of increases in blood lactate concentration during and following muscular activity. In contrast to Hill et al.'s (1923) demonstration of a constancy of blood lactate concentration during steady state exercise, Bang (1936) demonstrated that blood lactate concentration decreased during steady state exercise of both short and more prolonged duration. This finding was later confirmed by Flock et al. (Flock, Ingle, & Bollman, 1939) who stimulated rat muscles, and found rapid increases in lactate content during the first minute of stimulation, followed by a decline in lactate concentration whether the muscle continued to be stimulated or not.

These early investigations of the rise in blood lactate concentration as a consequence of muscular activity were supplemented by other investigations which determined the response of blood lactate concentrations to a period of training. Owles was perhaps the first person to describe the effect of training on the appearance of lactate in the blood (1930). He observed no increase in venous blood lactate concentration in two trained subjects, and attributed this

finding to only small amounts leaving the active muscles, or to its "dilution" in the general circulation as it passed through the inactive muscles of the forearm.

In one of the first training studies reported, Robinson and Harmon (1941) examined the effects of a 6 month, 4 day per week running training program on lactate metabolism. They found no changes in blood lactate concentration at rest following training, but noted a significant reduction in blood lactate concentration during submaximal exercise. Several other investigators, as summarized by Crescitelli and Taylor (1944), also noted that blood lactate concentrations were higher in untrained individuals during submaximal exercise.

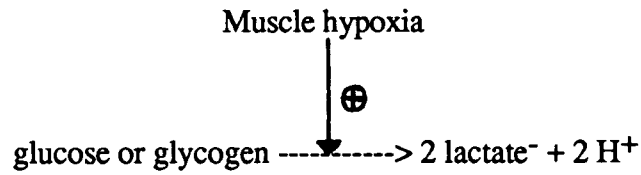
Until this time no definitive statement had been made on causes for the appearance of lactate in the blood. The reasons for the lower blood lactate concentrations observed during exercise as a consequence of training had yet to be described. The findings of these early experiments subsequently led to further in-depth investigations of lactate metabolism, and to the conclusion that the blood lactate concentration is dependent on rates of lactate production in the active muscle, rates of transport across the cell membrane, and rates of removal from the blood.

LACTATE PRODUCTION

Oxygen delivery limitations

Numerous investigations have shown that altering O_2 availability affects cellular metabolism during submaximal exercise. A reduced O_2 supply during submaximal exercise results in elevated blood lactate (Hughes, Clode, Edwards, Goodwin, & Jones, 1968; Knuttgen, & Saltin, 1973; Rowell, Blackmon, Kenny, & Escourrou, 1984; Woodson, Wills, & Lenfant,

1978) and muscle lactate concentrations (Katz, & Sahlin, 1987; Linnarsson, Karlsson, Fagraeus, & Saltin, 1974). Hill and Lupton (1923) were the first to suggest that local muscle hypoxia was responsible for the increase in blood lactate concentration observed during exercise. The central concept underlying this proposal was that an increase in lactate production was the result of an inadequacy of O_2 delivery to the active muscle mass, and that this increase in muscle lactate production caused an increase in blood lactate concentrations. This proposal was expanded by Bock and Dill (1931) who noticed that the apparently linear relationship between ventilation (V_I) and VO_2 is altered at high intensities of exercise. They attributed this phenomenon to muscle hypoxia resulting in rapid lactic acid formation at high intensities of exercise.



The VO_2 at which the rise in V_I appeared to deviate from linearity was subsequently termed the "anaerobic threshold" by Wasserman and his co-workers (Wasserman, & McIlroy, 1964; Wasserman, et al., 1973). They proposed a mechanism for the increase in blood lactate concentration observed with increasing work rate. They hypothesized that with increasing work rate, the rate of oxygen demand exceeds the rate of oxygen supply, due to increased metabolic activity in active muscle units and inadequate O_2 delivery to the active muscle. They hypothesized that, at some point, the oxygen supply becomes insufficient for ATP production by aerobic processes, and the ATP necessary to maintain muscle contraction needs to be produced anaerobically. The diminished O_2 availability results in an increase in lactate production in the active muscle.

The limiting factor therefore in aerobic ATP production is usually attributed to O_2 delivery. Consequently, lactate release from and accumulation in muscle is attributed to hypoxia in the tissue (Astrand, & Rodahl, 1977; Knuttgen, et al., 1973; Pedersen, 1983). When humans have been exposed to exercise under hyperoxic conditions (Astrand, 1954; di Prampero, 1981; Knuttgen, et al., 1973; Pedersen, 1983), the concentration of blood lactate at any given external work rate is lower than in normoxic conditions. These observations support the hypothesis that lactate formation is dependent on the availability of oxygen in the active muscle fibres.

This hypothesis that lactate formation during submaximal exercise is due to diminished O_2 availability has, however, been questioned. Measurements of cytosolic O_2 tension show that relatively high levels of O_2 are present in muscles that are producing lactate (Connett, Gayeski, & Honig, 1984). As recently reviewed by Walsh and Banister (1988), one prediction of the "inadequacy of muscle perfusion" hypothesis is that at a given work rate, O_2 uptake should be higher and hence blood lactate concentration lower in the trained person. It has however been shown that although blood lactate concentration is lower in the trained person at any absolute work rate, oxygen uptake is the same (Holloszy, 1973; Ivy, Withers, Handel, Elger, & Costill, 1980; Skinner, & McLennan, 1980) or may actually be lower (Yoshida, Suda, & Takeuchi, 1982).

A systematic investigation by Doll et al. (Doll, Keul, & Maiwald, 1968) of oxygen (PO_2) and carbon dioxide (PCO_2) pressure, pH, standard bicarbonate, and base excess in the arterial and venous blood of muscles during a pre-work period of rest, during steady-state exercise, and during recovery, revealed that the formation of lactate during exercise is not caused by O_2 insufficiency. In this investigation, the critical PO_2 for the muscle cell of 7.0 torr (Breitschneider, 1961), was not reached even during maximal exercise. Doll et al.

(1968) were able to demonstrate a significant increase in lactate concentration in the femoral venous blood at PO_2's of 23 torr, providing additional support for the belief that lactate can appear in the blood in the absence of muscle hypoxia. Chance and Quistorff (1978) have calculated that the minimum PO_2 required for maximum mitochondrial ATP production is between 0.1 and 0.5 torr. During supramaximal stimulation of dog gracilis muscle the PO_2 in the mitochondria is between 1.0 and 2.0 torr (Connett, et al., 1984).

The mitochondrial redox (NAD^+/NADH) state can be used as a reflection of oxygen availability in the mitochondria. Investigations of humans during exercise in which total cellular NAD^+ (Graham, Sjogaard, Lollgen, & Saltin, 1978) and total NADH have been determined (Graham, et al., 1978; Henriksson, Katz, & Sahlin, 1986; Sahlin, 1985; Sahlin, Katz, & Henriksson, 1987) suggest that the mitochondrial redox state is decreased, implying that the increased lactate production could be the result of a reduced availability of oxygen in the contracting muscle. Investigations showing a decline in total NAD^+ in humans exercising at VO_2max (Graham, et al., 1978), and large increases in total NADH with exercise requiring 75-100% VO_2max (Sahlin, 1985; Sahlin, et al., 1987), suggest a deficiency of O_2 in the mitochondria. This finding was recently substantiated by examining the NADH content in both Type 1 and Type 2 muscle fibre types sampled at exercise corresponding to 40, 75 and 100% VO_2max (Ren, Henriksson, Katz, & Sahlin, 1988). Increases in NADH concentration in both fibre types after exercise at 75% and 100% VO_2max suggested that O_2 availability was decreased at these high intensities of exercise. The increases in NADH concentration in both fibre types coincided with an accumulation of lactate, suggesting that the redox state of muscle is of importance for lactate formation.

However, these findings were recently questioned by Graham and Saltin (1989) from studies of humans who exercised for 5 minutes at 75% VO_2max, and then at 100%

VO_2max to exhaustion. They used the technique of employing the mass action ratio of glutamate dehydrogenase to estimate the mitochondrial redox state. This technique has the advantage of being independent of the bound components of NAD^+ and NADH, and also being independent of the extramitochondrial compartment, factors which Graham et al. (1978), and Sahlin et al. (1985; 1987) could not account for. They showed that despite high levels of muscle lactate accumulation, the estimated mitochondrial NAD^+-to-NADH ratio increased in both submaximal and maximal exercise.

The result of Graham and Saltin's investigation (1989) supports the suggestion of Brooks (1986a) and Saltin and Gollnick (1988) that active muscle increases its lactate production due to a mass action effect. Despite the high levels of muscle lactate accumulation in Graham and Saltin's investigation (1989), the estimated mitochondrial redox state rose 300% in both exercise bouts. This increase in mitochondrial redox state should increase the activity of key oxidative enzymes and promote increased VO_2, a requirement for sustained heavy exercise. In contrast, the decline in the mitochondrial redox state shown by Graham et al. (1978) and Sahlin et al. (1985; 1987), would have a large inhibitory influence on the activity of the mitochondrial oxidative enzymes, making it difficult to maintain adequate Krebs cycle flux and consequently VO_2 to sustain high intensity exercise.

Further evidence for lactate accumulation occurring in the absence of local tissue hypoxia has also been demonstrated in electrically stimulated muscle (Jobsis, & Stainsby, 1968; Stainsby, Brechue, O'Drobinak, & Barclay, 1989) and vascularly isolated autoperfused muscle (Connett, et al., 1984; Connett, Gayeski, & Honig, 1986). In the latter experiments, when type I muscle was stimulated at 10-100% VO_2max, tissue lactate concentrations increased with increasing VO_2, but was unaffected by tissue PO_2. A marked lactate efflux was also observed during stimulation above 50% VO_2max. The

conclusions derived from Connett et al.'s (1984; 1986) investigations were that a) lactate efflux in isolated Type 1 muscle increased markedly above a threshold work rate, b) at this threshold, VO_2 was not limited by O_2 delivery, and neither hypoxic nor anoxic loci were found, c) at all work rates including those near VO_2max, lactate efflux was not a simple function of intracellular PO_2, d) lactate efflux was not linearly correlated with lactate concentration [La], and e) [La] was related to VO_2 but not PO_2. The authors concluded that neither tissue lactate nor blood lactate concentrations can be used to impute muscle O_2 availability or glycolytic rate.

Several studies have shown that non-muscle tissues can release significant amounts of lactate into the systemic circulation under resting normoxic conditions, by converting glucose to lactic acid. (Bartels, Vogt, & Jungerman, 1988; Newgard, Hirsch, Foster, & McGarry, 1983; Smadja, Morin, Ferre, & Girard, 1988; Wilson, 1956). Foster (1984), and Newgard et al. (1983), have shown that liver glycogen synthesis can occur via an "indirect pathway" whereby dietary carbohydrate is digested to glucose, and glucose is then catabolized to lactic acid. This lactic acid then serves as a precursor for liver glycogen synthesis. The importance of an indirect pathway of liver glycogen synthesis after eating, and the formation of lactic acid by non-muscle tissue under resting normoxic conditions, is that tissue hypoxia cannot be regarded as the sole source of lactic acid production.

The rate of lactate production is dependent on the kinetics of glycolysis, the activity of lactate dehydrogenase (LDH), and mitochondrial respiration. From the studies reviewed above, it is unlikely that a diminished O_2 availability to the mitochondria is the primary stimulus for the increased lactate appearance in the blood during heavy exercise, and certainly not during submaximal exercise. Lactate accumulates in muscle and blood in the presence of an adequate PO_2, and when the mitochondrial redox state is markedly elevated.

Substrate Utilization

An important determinant of the capacity for continuous muscular work is the quantity of glycogen stored in the muscle (Ahlborg, Bergstrom, Ekelund, & Hultman, 1967; Bergstrom, Hermansen, Hultman, & Saltin, 1967a; Bergstrom, & Hultman, 1967b). These investigations have demonstrated that exercise at a high work intensity cannot be continued when the muscle glycogen store is almost depleted.

The importance of muscle glycogen as a primary substrate for lactate production has been demonstrated in patients with McArdle's disease who lack the enzyme glycogen phosphorylase (or other enzymes) in their skeletal muscles (Hagberg, et al., 1982). During the most intense exercise that these individuals can perform, there is no release of lactate from the active muscle. However, muscle uptake of infused glucose is possible, and the pyruvate which is produced can be transported into the mitochondria for terminal oxidation to CO_2 and H_2O. No measurable lactate production occurs with the infusion of glucose in McArdle's patients. The increased glucose availability allows these patients to perform moderate exercise. In addition, in normal subjects a reduced blood lactate concentration has been observed when muscle glycogen is decreased, such as in fasting or intense exercise (Asmussen, Klausen, Nielsen, Techow, & Tonder, 1974; Astrand, Hallback, Hedman, & Saltin, 1963; Karlsson, 1971; Saltin, & Hermansen, 1967). These observations suggest that lactate production is dependent on the amount of glycogen stored in the muscle and on activation of glycogenolysis and glycolysis.

From their investigations, Pande (1971) and Paul (1970) suggested that glycolytic flux and lactate production were partly dependent on the concentration of free fatty acids (FFA) available to the muscle cell for oxidation. Essen et al. (1975b) proposed that net lactate

accumulation during exercise could depend on an imbalance in the availability of carbohydrate and fat. A large amount of data support the theory that biochemical adaptations to training improve the trained muscles' capacity for fat oxidation (Gollnick, Riedy, Quintinskie, & Bertocci, 1985; Henriksson, 1977; Saltin, & Karlsson, 1971a; Saltin, & Karlsson, 1971b). The lower muscle and blood lactate concentrations observed in trained individuals is attributed to this enhanced fat oxidation (Favier, Constable, Chen, & Holloszy, 1986; Henriksson, 1977; Saltin, et al., 1971a; Saltin, et al., 1971b). Increased fat mobilization increases citrate formation. Increases in resting levels of citrate in muscle have been observed following prolonged exercise and a diet rich in fat (Essen, Hagenfeldt, & Kaijser, 1977; Jansson, 1980).

The phosphofructokinase (PFK) reaction is inhibited in vitro by citrate (Garland, Randle, & Newsholme, 1963) and is proposed to be a regulator of glycolytic rate in the resting muscle (Newsholme, & Start, 1979). Inhibition of PFK via citrate would decrease the rate of glycolysis in trained individuals during exercise, thereby decreasing lactate formation. Whether such regulation of PFK by citrate, as observed in resting muscle, occurs also during exercise is speculative since, as recently described by Connett (1987), the regulation of PFK activity is complex and is modulated by pH, $MgATP^{2-}$, $MgADP^{-}$, citrate, AMP, NH_4^+, and hexose biphosphates as well as enzyme phosphorylation and calcium.

Lower blood lactate concentrations due to an enhanced capacity for fat oxidation subsequent to training, may be due not only to an increased availability of plasma FFA, but also to an increased lipoprotein lipase (LPL)-activated hydrolysis of plasma triglycerides (Robinson, 1970). An increase in plasma LPL activity has also been observed following prolonged exercise, with the duration of the exercise being an important determinant of LPL activity (Lithell, Orlander, Schele, Sjodin, & Karlsson, 1979; Taskinen, Nikkila, Rehunen, &

Gordin, 1980). Hurley et al. (1986) showed an increased triglyceride lipolysis in muscles of trained compared to untrained humans. The mechanism responsible for the increased lipolysis was possibly due to greater activity of hormone sensitive lipase in muscle, as had been shown previously in trained rats (Oscai, Caruso, & Wergeles, 1982).

Improvements in the rates of fat oxidation as a result of endurance training, have been assessed using the ratio of CO_2 produced/O_2 consumed (termed the respiratory quotient, RQ or respiratory exchange ratio, RER). Both the RQ and RER are significantly lower in the trained state, indicating a greater reliance on fat oxidation during exercise following training (Henriksson, 1977; Saltin, et al., 1971a). It has also been established that blood lactate concentration can be decreased at any given external work rate by increasing the FFA concentrations in the blood (Costill, et al., 1977; Costill, Dalsky, & Fink, 1978). The relationship between plasma FFA concentration and the rate of plasma FFA oxidation is usually linear (Hagenfeldt, 1975; Paul, 1970). Hurley et al. (Hurley, et al., 1986) have shown that following training, plasma FFA concentrations are lower during submaximal exercise, suggesting that the rate of uptake into muscle and oxidation of plasma FFA may be increased in the trained state. However, a similar training program resulted in decreased catecholamine levels during submaximal exercise (Winder, Hickson, Hagberg, Ehsani, & McLane, 1979). The lower plasma FFA concentrations could therefore have been due to a reduced catecholamine drive. With significantly greater lipolysis of muscle triglycerides after training, and a 65% increase in the amount of energy derived from fat (calculated from the RER), Hurley et al. (1986) concluded that training results in an increased utilization of fatty acids derived from source other than plasma FFA.

In a separate investigation, Hurley et al. (Hurley, et al., 1984) have also shown that plasma lactate concentrations at the same work rate, and also at the same relative exercise intensities

in the range of 55 to 75% VO_2max, are significantly lower following training. It could therefore be implied that the greater utilization of lipid as a fuel could have a sparing effect on the use of muscle glycogen, resulting in a lower concentration of plasma lactate following training.

An RER which approaches unity implies an increased rate of CHO oxidation and hence lactate formation. The observation of a lower RER and reduced blood lactate concentrations during exercise, suggests a reduced rate of CHO oxidation and consequently lactate formation. Use of the RER as a measure of the relative rates of substrate utilization during exercise has been questioned by Jones et al. (1980). They investigated the metabolic effects of light and heavy exercise, and showed that excess carbon dioxide may be evolved in exercise which elicits constant plasma lactate concentrations. Because of this observation, they suggested that changes in the RER may not give valid information regarding substrate usage, except in exercise of long duration, when plasma lactate and bicarbonate concentration do not change from resting values. The use of lower RQ or RER values as indicators of decreases in CHO oxidation(and hence blood lactate levels) is therefore questionable, since the finding of a high RER may not exclude the use of fat as a major fuel source in exercise associated with lactate production (Jones, et al., 1980).

The lower blood lactate concentrations seen after endurance training are therefore likely partly due to changes in substrate use. Increases in muscle mitochondrial mass and the activity of mitochondrial enzymes, allowing for greater ATP production via oxidative processes, would decrease glycolytic flux and thus lactate formation. The improvement in fat oxidation as a result of training would decrease the requirement for ATP formation via glycolysis, and reduce the rate of production of lactate.

Muscle Fibre Recruitment

Sherrington was the first to propose a definition of the motor unit as the quantum of motor system output, and the idea of recruitment to describe the gradation of total muscle force by addition and subtraction of active motor units (Eccles, & Sherrington, 1930; Liddell, & Sherrington, 1925). Denny-Brown, a student of Sherrington, demonstrated that preferential recruitment of fast twitch white muscles could occur under certain conditions (Denny-Brown, 1929). A major advance in the understanding of recruitment patterns in different muscle fibre types, innervation of different muscle fibre types and functional thresholds of motor unit recruitment, was made by Henneman and his co-workers at Harvard (Henneman, & Olson, 1965; McPhedran, Wuerker, & Henneman, 1965; Wuerker, McPhedran, & Henneman, 1965). These investigations lead to the well-known "size principle" of motor pool organization attributed to Henneman.

Not only are the muscle fibre types distributed in regular patterns in the muscles, but they also appear to be recruited in reasonably predictable spatial patterns during exercise. The most direct evidence indicating the general patterns of recruitment of the fibres in conscious animals during locomotion comes from electromyographic (EMG) measurement (Armstrong, & Laughlin, 1985a).

Clausen (1976) proposed that the increase in lactate concentration that occurs during incremental exercise is due to the progressive recruitment of larger motor units according to the size principle of Henneman (Henneman, 1957; Henneman, et al., 1965). There is good evidence in animal studies of a progressive activation of motor units from slow to fast muscles with increasing running speed (Gardiner, Gardiner, & Edgerton, 1982; Smith, Edgerton, Betts, & Collatos, 1977; Walmsley, Hodgson, & Burke, 1978).

The EMG method for evaluating possible associations between muscle fibre recruitment patterns and lactate production was used by Nagata et al. (Nagata, Muro, Moritani, & Yoshida, 1981), to establish a connection between a non-linear integrated EMG (IEMG) increase, and increases in blood lactate concentrations during incremental cycle ergometry. These investigators demonstrated that the observed increase in blood lactate concentration during incremental exercise is due to recruitment of fast twitch fibres and an increase in the firing frequency of fast twitch fibres already recruited.

Changes in blood lactate concentrations during submaximal exercise are due to changes in recruitment of muscle fibre types (Jacobs, 1981). In addition, the intensity of exercise at which blood lactate begins to accumulate is related to the percentage of slow twitch muscle fibres (Tesch, Sharp, & Daniels, 1981). Because of their large motoneurons and high glycogenolytic enzyme and myofibrillar ATPase activities (Essen, Jansson, Henriksson, Taylor, & Saltin, 1975b; Sjodin, 1976), it is to be expected that increased lactate production would accompany recruitment of fast twitch muscle fibres.

Bauer et al. (1986) reported that fast twitch glycolytic fibres produce lactate exclusively as an end-product of glycogen degradation, whereas glucose is catabolized to both lactate and CO_2. Since fast glycolytic fibres have a low oxidative capacity, this finding would suggest that an increase in lactate production would occur when they are activated. The greater increase in lactate accumulation in fast twitch vs. slow twitch muscle fibres in exercise to exhaustion, would tend to support the relationship between percentage muscle fibre type activated and the rate of lactate production (Tesch, Sjodin, & Karlsson, 1978).

It can therefore be concluded that a relatively orderly recruitment of muscle fibres occurs during incremental exercise. As recently studied by Helal et al. (1987), EMG analysis

suggests that the recruitment pattern of skeletal muscle in incremental exercise is due to the progressively increasing number of fast motor units activated rather than a sudden activation of all these fibres. This would be compatible with a non-threshold, exponential increase in the rate of lactate production during incremental exercise. Based on this review of the literature, increased blood lactate concentrations would be expected to be at least in part due to changes in recruitment patterns of skeletal muscle fibres during heavy exercise.

Catecholamines

During exercise there is an increase in both plasma epinephrine (E) and norepinephrine (NE) concentrations. Generally, changes in NE concentrations are related to hemodynamic parameters and oxygen requirement, while plasma E concentrations are dependent on direct (sympathetic nerve activity) and indirect (blood glucose concentration) effects. Increases in plasma NE during exercise have been described by von Euler (1956; 1961), with no significant differences observed between trained and untrained subjects working at the same work rate (Vendsalu, 1960). An earlier investigation by von Euler and Helner (1952) showed that the NE concentration in the urine appeared to be a function of the work rate measured as a percentage of the individual's VO_2max. Haggendal et al. (1970) determined the NE response to incremental exercise in trained and untrained men. Up to 75% VO_2max NE levels increased slowly, but then increased rapidly in a curvilinear fashion. These investigations were not able to demonstrate any detectable increase in E in the blood, and thus supported the finding that NE concentrations are a function of the intensity of muscular work.

More recent investigations have demonstrated increases in plasma E and NE during incremental exercise, with only slight rises in these hormones at exercise intensities up to

~ 50% VO_2 max (Keul, Dickuth, Simon, & Lehmann, 1981; Peronnet, Nadeau, Champlain, Magrassi, & Chatrand, 1981). At work rates higher than 50% VO_2max, blood lactate (Campbell, et al., 1989; Hughson, et al., 1987) and plasma E and NE increase in a curvilinear fashion (Haggendal, et al., 1970; Keul, et al., 1981; Peronnet, et al., 1981).

Vagal tone increases as a result of endurance training (Ekblom, Kilbom, & Soltysiak, 1973), and decreases in sympathetic activity are seen at the same workloads during exercise after training (Lehmann, Keul, Huber, & Prada, 1981; Peronnet, et al., 1981; Winder, et al., 1979). These changes in catecholaminergic discharge are associated with reduced plasma lactate concentrations. Stainsby et al. (1984) studied the effect of E and NE infusion on rates of muscle lactate production, using an in situ gastrocnemius-plantaris muscle preparation stimulated at a constant rate. This investigation revealed that both E and NE increased maximal net lactate production during contractions, but that arterial and venous lactate concentrations increased continuously during E infusion and remained constant during NE infusion. These investigators also determined that E and NE did not have any measurable effect on rates of lactate output after 10 minutes of contraction. The probable explanation of this finding is firstly that the contractions were performed at a constant rate, thus probably allowing the muscles to derive their energy from source other than glycolysis. Secondly, the pattern and quantity of lactate exchange that would have occurred if E and NE had varied as they do in exercise was not investigated (Stainsby, et al., 1984). Important additional findings in this study were that inactive muscles may make a major contribution to blood lactate concentration during E infusion, and that plasma E concentration is an important variable in the consideration of factors determining plasma lactate concentration even at rest.

In a study to determine whether the in vivo effect of E on glycogenolysis is exerted directly on the muscles or indirectly by depressing insulin secretion, Richter et al. (1982) electrically stimulated an isolated perfused rat hindquarter at subtetanic threshold for 20 minutes. During stimulation they investigated the effects of E infusion. They concluded that muscle glycogenolysis during exercise was under dual control of contractions and E; contractions were the primary cause of glycogenolysis early in exercise, and a direct effect of E was necessary to maintain continued glycogenolysis during the contractions. In addition, through infusion of E into exercising dogs, it has been shown that Beta stimulation activates glycogenolysis and lactate formation (Issekutz, 1984; Issekutz, & Allen, 1972).

It is possible that rises in E during exercise could raise blood lactate concentrations by decreasing blood flow through the hepatic-splanchnic areas which are sites of lactate removal. Gregg et al. (1989) observed in exercising anemic rats, that elevated catecholamines were associated with shunting of blood away from the liver and kidneys and a decreased rate of lactate clearance from the blood . A significant decrease in kidney and splanchnic blood flow at high running speeds in rats, when catecholamine levels were high, was also reported by Armstrong and Laughlin (1985b).

The results of the above investigations suggest an important role of catecholaminergic stimulation in both lactate production and lactate removal.

LACTATE TRANSPORT

Simple diffusion of lactate across the sarcolemmal membrane has been shown in human erythrocytes (Deuticke, Beyer, & Forst, 1982; Dubinsky, & Racker, 1978) where it accounts for approximately 5% of the total lactate efflux, and in toadfish hepatocytes where

it accounts for all of the transfer of lactate (Walsh, 1987). The importance of a carrier-mediated mechanism was shown in rat hepatocytes in which diffusion accounted for only 20% of the lactate flux, with most of the transport occurring via a facilitated carrier-mediated process for lactic acid or lactate (Fafournoux, Demigne, & Remesey, 1985). More recent work using rat skeletal muscle has provided additional evidence for a skeletal muscle sarcolemmal lactate transporter (Roth, et al., 1990a; Roth, et al., 1990b).

The presence of a lactate transporter located in the cell membrane was first demonstrated in bacterial cells, and then later studied in detail in mammalian tumour cells (Spencer, & Lehninger, 1976) and human red blood cells (Dubinsky, et al., 1978). As described by Deuticke (1980), this transporter is often referred to as the organic anion or substituted monocarboxylate transporter because of its generic carrier properties, whose main function is to export lactate. This transporter appears to be a membrane protein that co-transports a lactate anion and a proton externally without a requirement for ATP (Dubinsky, et al., 1978; Spencer, et al., 1976).

In experiments investigating lactate transport in human erythrocytes, Deuticke et al. (1982) determined that the lactate transporter is responsible for approximately 90% of lactate efflux, with the remainder being removed from the cell by diffusion and the chloride bicarbonate exchanger. With increasing lactate concentration, such as may occur in skeletal muscle during intense physical exercise when the rate of metabolism of lactate in skeletal muscle may be low, the transport of lactate across the sarcolemmal membrane may become saturated. It has been suggested that this saturable transmembrane movement of lactate may occur in skeletal muscle with increases in lactate concentration (Eggleton, Eggleston, & Hill, 1928; Hirche, Hombach, Langohr, & Wacker, 1972; Hirche, Hombach, Langohr, Wacker, & Busse, 1975; Jorfeldt, Juhlin-Dannfelt, & Karlsson, 1978; Karlsson, 1971; Karpatkin,

Helmreich, & Cori, 1964). However, the results of these investigations are not as definitive as they may seem due to the existence of extracellular diffusion restraints in whole-muscle preparations. Koch et al. (1981) described the limitations of these early experiments by indicating that if the time-course for transmembrane movement is rapid compared with that for transendothelial passage, distorted results can be obtained. In addition, if there is progressive vasodilatation during the time that cellular lactate concentrations are rising, a completely passive system would appear to exhibit saturation kinetics.

To bypass these limitations, Koch et al. (1981) devised a method of transient analysis to investigate the nature of lactate transfer across mouse diaphragm cells. The results of their investigation indicated that lactate crosses the cell membrane both by free diffusion and by a carrier system. Approximately three-quarters of the transport at low lactate concentrations was carrier-mediated, with saturation of this mechanism occurring at ~ 10 mmol. l^{-1}. Transport of lactate using this system is bidirectional and acts as a facilitated diffusion rather than an active transport process.

A more recent investigation by Watt et al. (1988) confirmed the findings of Koch et al. (1981) but also found that trans-sarcolemmal lactate transport is pH-dependent. This investigation showed that mixed mammalian skeletal muscle is capable of exchanging approximately 10% . min^{-1} of lactate (0.5 to 50 mM) over the physiological concentration range with little interference by saturation of any carrier-mediated mechanism. This investigation also showed that lactate flux showed increases of 30-35% with a fall of 0.6 pH units. Such pH changes have been demonstrated as a result of strenuous exercise (Sahlin, Harris, Nylind, & Hultman, 1976), and thus a decreased intracellular muscle pH would increase the rate of lactate efflux.

The distribution of lactate across the cell membrane of rat diaphragm muscle was reported by Roos (1975) to be in accordance with the transmembrane gradient, supporting the suggestion that lactate crosses the diaphragm membrane in conjunction with protons. By establishing correlations between changes in the pH of the blood or skeletal muscle and the concentrations of lactate measured simultaneously in the same samples, it was concluded that the amount of lactate present is equivalent to the surplus amount of hydrogen ions (H^+) present in the same sample. For example, Hirche et al. (1975) showed that in alkalosis, H^+ penetrates as rapidly as lactate into the blood perfusing electrically stimulated muscle of the dog. In the same experiments H^+ ions were found to permeate even more slowly than lactate during the first 3-4 minutes of exercise.

Mainwood and Worsley-Brown (1975) reported similar time courses of lactate and H^+ efflux in superperfused frog sartorius muscles during recovery after stimulation in vitro. These investigators were unable to suggest why their findings differed from those of Heisler (1973) who demonstrated different efflux rates for lactate and hydrogen ions from rat diaphragm in vitro. Supporting evidence for Heisler's work came from studies of isolated rat diaphragms and frog sartorius muscles, in which Benade and Heisler (1978) showed that the rate of efflux of H^+ ions exceeded that of lactate by factors of approximately 14 and 50 in the case of diaphragm and sartorius muscles respectively. Based on these findings, they concluded that the difference observed in efflux kinetics of H^+ and lactate ions, indicates that the lactate content of a body compartment does not represent the absolute hydrogen ion load of the same compartment, particularly during the early phase of the efflux process.

Juel (1988) examined intracellular pH recovery and lactate efflux in mouse soleus muscles stimulated in vitro. The major finding of this investigation was the involvement of a Na^+/proton exchange system and a lactate carrier system in the sarcolemma of mammalian

skeletal muscle. Following muscle activity it was demonstrated that the lactate carrier mechanism accounted for more than half of the lactate efflux. Juel (1988) also showed that the intracellular pH recovery is accelerated by activation of Na^+/proton exchange. He found that amiloride, an inhibitor of Na^+/H^+ exchange , inhibited the rate of intracellular pH recovery.

Mason and Thomas (1988) confirmed the findings of Koch et al. (1981), Hirche et al. (1972), Mainwood and Worsley-Brown (1975), Hirche et al. (1975) and Mason et al. (1986) by showing that lactate and proton equivalents cross the membrane together. They also confirmed the work of Roos (1975) who showed that lactate is distributed across the cell membrane in accordance with the transmembrane pH gradient. In addition, Mason and Thomas (1988) are in agreement with Koch et al. (1981) that uptake of lactate involves both a saturable and a non-saturable component, and in the physiological range of lactate concentrations and pH, the transport process dominates. The recent work of Roth and Brooks showed that the skeletal muscle lactate transporter demonstrates saturation kinetics, competition, stereospecificity, and sensitivity to temperature as well as various ionic *cis*-inhibitors (1990a; 1990b). Furthermore, the lactate transporter is also a potentially important regulator of lactate flux across skeletal muscle, and may help to regulate intracellular pH and intermediary metabolism during conditions eliciting high lactate concentrations.

It is, however, unknown whether endurance exercise training has any effect on the lactate transport mechanism.

LACTATE REMOVAL

The traditional concept of how the lactate produced during exercise is removed, can be attributed to A. V. Hill who first described the "O_2 debt" hypothesis (Hill, et al., 1925), the name given to the recovery oxygen consumption. This hypothesis suggested that the accumulation of blood lactate during the anaerobic component of exercise represented a stored energy "credit", glycogen, which following cessation of exercise, needed to be repaid - the "debt". This repayment of debt was believed to serve two functions, namely, the replenishment of the original carbohydrate stores by resynthesizing approximately 80% of the lactate back to glycogen in the liver or directly in muscle, and to oxidize the remaining lactate following its conversion to pyruvate in the mitochondria. Subsequent to the work of Hill et al., Margaria et al. (1933) showed that the initial portion of the oxygen "debt" was repaid before blood lactate concentrations began to decrease. They proposed two phases of O_2 debt; an alactic or alactacid oxygen debt, attributed to the restoration of the ATP and CP concentrations depleted during exercise, and a lactacid debt whereby a major portion of the O_2 debt was thought to represent the reconversion of "lactic acid" to glycogen in the liver and muscle.

To prove the speculation of Hill et al. (1925) and Margaria et al. (1933) it must be established that the major portion of the lactate produced during exercise is resynthesized to glycogen in recovery. This has never been shown (Brooks, & Gaesser, 1980). Since most of the glycogen mobilized during exercise is oxidized during the exercise itself, it is not possible to restore 80% of the glycogen catabolized from lactate. Futhermore, injections of radioactive labeled U-[^{14}C]-lactate into rats exercised to exhaustion, has shown that only around 20% of the formed lactate is converted into glycogen after exercise (Brooks, et al.,

1980). Most of the lactate formed during exercise undergoes oxidation by cardiac and skeletal muscle to CO_2.

The removal of lactate during exercise was investigated by Newman et al. (1937), who confirmed previous reports that the rate of removal of lactate from the body during exercise is a logarithmic function of time. These investigators also determined that the rate of removal of lactate was greater when moderate exercise was performed, than when the subjects rested after exercise, supporting the concept of a balance between lactate formation and removal. They further concluded that the rate of removal of lactate increases approximately proportionately with the metabolic rate up to some critical level of activity, this critical level being different for each subject. The possible fates of the lactate produced during exercise were suggested to be removal through sweat and urine, oxidation by the heart, and resynthesis to glycogen in skeletal muscle.

Removal via Gluconeogenesis

Raising blood lactate concentrations, due to either lactate infusion (Cori, & Cori, 1929) or exercise (Himwich, Koskoff, & Nahum, 1930), have shown that a large fraction of the lactate present in the blood is removed by the liver. Other sites implicated in lactate removal during exercise are the heart (Carlsten, Hallgren, Jagenburg, Svanborg, & Werko, 1961), the kidney (Krebs, 1964), resting skeletal muscle (Jorfeldt, 1970), and exercising skeletal muscle (Hermansen, et al., 1973).

Rowell et al. (1966) investigated the significance of human hepatic-splanchnic tissues in removing lactate from blood during prolonged mild to heavy exercise requiring 48 to 70% of VO_2max. They concluded that up to 50% of the lactate produced may have been

removed via gluconeogenesis by hepatic-splanchnic tissues at this intensity of exercise. In contrast to this finding, Ahlborg et al. (1976) estimated that only 15% of an infused lactate load was accounted for by splanchnic uptake during cycle ergometer exercise at 31% of VO_2max; approximately 56% of the administered lactate was accounted for by muscle uptake and dilution. Hepatic gluconeogenesis in these investigations was calculated from measurements of hepatic blood flow and arteriovenous lactate differences.

Kreisberg et al. (1970) investigated the conversion of labeled lactate to glucose using isotope dilution techniques at rest. They concluded that 15% of the glucose turnover rate in the normal postabsorptive human is derived from lactate. This finding had previously been shown in studies of lactate/glucose interconversion, where indirect estimates of lactate conversion to glucose yielded values of 15 to 25% (Kreisberg, 1968; Reichard, Moury, Houchella, Patterson, & Weinhouse, 1963).

Radioisotope dilution experiments in humans during exercise are few in number, with little attention having been given to the interactions between lactate and glucose kinetics. Until recently, radioisotopes had not been used to determine gluconeogenesis from lactate. Stanley et al. (1988) studied healthy men during exercise lasting from 30 to 50 minutes at 40% of VO_2max while they received an infusion of labeled glucose and lactate. These investigators determined that the estimated rate of gluconeogenesis represented 24% of the rate of lactate disappearance during exercise at this intensity. Using the values of Ahlborg et al. (1976) and Rowell et al. (1966) which were obtained using arterial-venous differences and hepatic blood flow, Stanley et al. (1988) calculated that gluconeogenesis in those investigations accounted for approximately 10 to 25% of the lactate disappearance rate. These values are similar to the values calculated from glucose labeled by ^{14}C-lactate in the subjects who received lactate infusions in Stanley's experiment.

Therefore, it appears that gluconeogenesis accounts for 20 to 30% of the lactate removal at low intensities of exercise in which blood lactate concentrations are not markedly elevated. The effects of endurance training on gluconeogenesis from lactate during exercise have, until now, not been determined in humans.

Removal via Oxidation

As described previously, lactate can be removed from the blood by several tissues, including the liver, heart, skeletal muscle and kidney. Although the quantity removed and the metabolic pathway utilized may vary in different tissues, there is good evidence that the fate of blood lactate is primarily one of oxidative removal during exercise (Minaire, 1973).

The heart has been known as a site of lactate oxidation for a number of years, with several investigations illustrating the removal of lactate by this organ (Gertz, et al., 1981; Keul, 1971). The extent to which human skeletal muscle could act in lactate removal was not known until Jorfeldt (1970), using radioisotopes, demonstrated that skeletal muscle is capable of taking up and directly oxidizing lactate. His investigation using forearm exercise in man, demonstrated that approximately 45% of infused lactate was oxidized. Stanley et al. (1985; 1986), also using radioisotope infusion in man, recently showed that the exercising legs are responsible for approximately 50% of the lactate cleared from the blood during exercise of moderate intensity. The findings of these experiments are supported by those of Mazzeo et al. (1986), suggesting that the primary fate of lactate removal in man is via oxidation. In these studies, it was estimated that up to 75% of the lactate formed during submaximal exercise, is removed via oxidation. The site of this oxidation seems to be localized to the slow-twitch (ST) fibres of human skeletal muscle. These fibres contain

approximately twice as much H-LDH as M-LDH (Sjodin, 1976), indicating that the oxidation of lactate to pyruvate would be favoured here.

The results of these investigations provide support for the lactate shuttle hypothesis proposed by Brooks (Brooks, 1985). This hypothesis suggests that lactate formed in some muscle fibres in which the glycolytic rate is high, is released into the interstitium and circulation and is shuttled to, taken up, and combusted by other muscle fibres with high rates of cellular respiration (Brooks, 1986a; 1986b).

These studies show the fate of lactate produced during submaximal exercise to be primarily one of oxidation. How endurance training affects the rates of oxidation of lactate during exercise in humans has, until now, not been determined.

The Lactate Threshold

In the early 1900's Fletcher and Hopkins (1907) investigated the metabolism of amphibian skeletal muscle. They demonstrated that a) resting muscle contained very little lactic acid, b) mechanical injury or irritation could increase the lactic acid concentration in the muscle, c) lactic acid can be produced by viable muscle under anaerobic conditions, d) fatigue resulting from contraction of the muscle was associated with an increase in lactic acid concentrations and e) hyperoxia depressed lactic acid formation. Their description of lactic acid as a metabolic product of living tissue, provided a basis for subsequent investigations leading to the development of the lactate threshold concept.

The work of Fletcher and Hopkins was substantiated by Ryffel (1909), who demonstrated an increased lactate concentration in the blood and urine of man following intense muscular

activity. One of the first descriptions of the gas exchange consequences of severe exercise and lactic acid formation in the muscles was provided by Christiansen et al. (1914). They showed that in severe muscular work, the H^+ released in the dissociation of lactic acid combines with bicarbonate, ultimately resulting in the formation of CO_2 in excess of that produced by the increased metabolic activity. The decreased bicarbonate concentration in the blood together with the increased CO_2 content, would stimulate the respiratory centre.

Owles (1930) provided the first account of the blood lactic acid changes that occur for a range of exercise intensities. The results of his study were a) there is some critical metabolic level below which lactic acid does not increase and above which it does increase, b) this critical metabolic level varies between individuals and mode of exercise and, c) the CO_2 combining power of the blood is reduced at exercise intensities above this critical level but not below it. Subsequently, Margaria et al. (1933) demonstrated that in individuals performing treadmill exercise, no marked elevation in blood lactate concentration occurs until a work rate of approximately two-thirds of maximum is reached, after which blood lactate concentrations increases rapidly. These studies thus provided the first evidence for a possible lactate threshold, in which blood lactate accumulation suddenly rises during incremental exercise.

In 1964, Wasserman and McIlroy presented the first definition of the lactate threshold. They made breath-by-breath measurements of RER and measurements of blood lactate concentrations over a wide range of exercise intensities. These measurements were used to determine the balance between O_2 supply and demand in exercising muscle. With increasing exercise intensities, lactic acid formation in muscle increases and bicarbonate buffering of H^+ dissociated from lactic acid increases release of CO_2, resulting in an increase in the RER. The point at which the bicarbonate concentration in the blood

decreased and blood lactate concentration increased, was termed the "anaerobic threshold". This lactate threshold definition was subsequently modified by Wasserman et al. (1973), to define the level of work or O_2 consumption just below that at which metabolic acidosis and the associated changes in gas exchange occur. The theoretical basis for this revised definition is that the lactate concentration in the blood increases to a sufficient point that the buffering of the associated H^+ by HCO_3^- would cause an increased CO_2 output, thereby increasing the chemoreceptor drive to the respiratory centre, leading to a non-linear increase in V_I with increasing VO_2.

This "anaerobic threshold" model of Wasserman et al. (1973) has proved to be particularly attractive to exercise physiologists, since it presented the possibility of detecting non-oxidative muscle metabolism by changes in breathing. However, factors other than an increased VCO_2 can contribute to the non-linear increase in V_I with increasing VO_2. During high intensity exercise, local hypoxia may be more pronounced in the peripheral chemoreceptor area, causing stimulation of the peripheral chemoreceptors with a resulting increase in V_I that has nothing to do with blood lactate concentrations (Whalen, & Nair, 1975). Furthermore, patients with McArdle's disease who, due to a lack of phosphorylase cannot utilize glycolysis and produce lactate, demonstrate a non-linear increase in V_I with increasing VO_2 during exercise (Hagberg, et al., 1982).

This concept continues to generate controversy, mainly because of the idea that the lactate threshold is due to inadequate O_2 delivery resulting in muscle hypoxia. The studies reviewed earlier, which provide substantial evidence that lactate production can occur when muscle receives adequate O_2, and that PO_2 is unlikely to fall to a critically low level during exercise, discount this threshold concept. In both muscle and blood, lactate has been shown to rise as a continuous, rather than as a threshold, function of VO_2 during incremental

exercise (Campbell, et al., 1989; Dennis, et al., 1991; Hughson, et al., 1987). These investigators showed that blood lactate thresholds are artifacts of an inappropiate use of linear equations to define an exponential process. Blood lactate concentration increases during incremental exercise are likely to be a complex function of many factors, rather than a sudden threshold response to inadequate O_2 delivery.

In summary, the accumulation of blood lactate during exercise is the consequence of factors influencing it's production, transport and removal. The catecholamines play an important role in both the stimulation of lactate production and in it's removal from the circulation. Although hypoxia can increase lactate production, the studies of Connett et al. (1984) suggest that this factor does not contribute to the increased blood lactate concentrations seen during exercise. The recruitment of fast glycolytic muscle fibres probably contributes to the rise in lactate accumulation during heavy exercise . Changes in substrate use, primarily, the improvement in fat oxidation as a result of endurance training, likely reduces the rate of production of lactate by decreasing the requirement for ATP formation via glycolysis. The lactate transporter controls the exchange of lactate between cells and tissues, and may contribute to the regulation of pH and intermediary metabolism during conditions of high lactate concentrations. Once formed, lactate can be "shuttled" to various tissues to be either oxidized or to act as a gluconeogenic precursor. Oxidation appears to be the primary fate of the lactate formed during exercise. Finally, the "anaerobic threshold" hypothesis of blood lactate accumulation continues to enjoy support; this despite the inappropiate use of linear equations to define an exponential process, as well as substantial evidence suggesting a complex interplay of many factors contributing to lactate accumulation.

A summary of the probable causes of blood lactate accumulation during progressive exercise is shown in figure 2.1

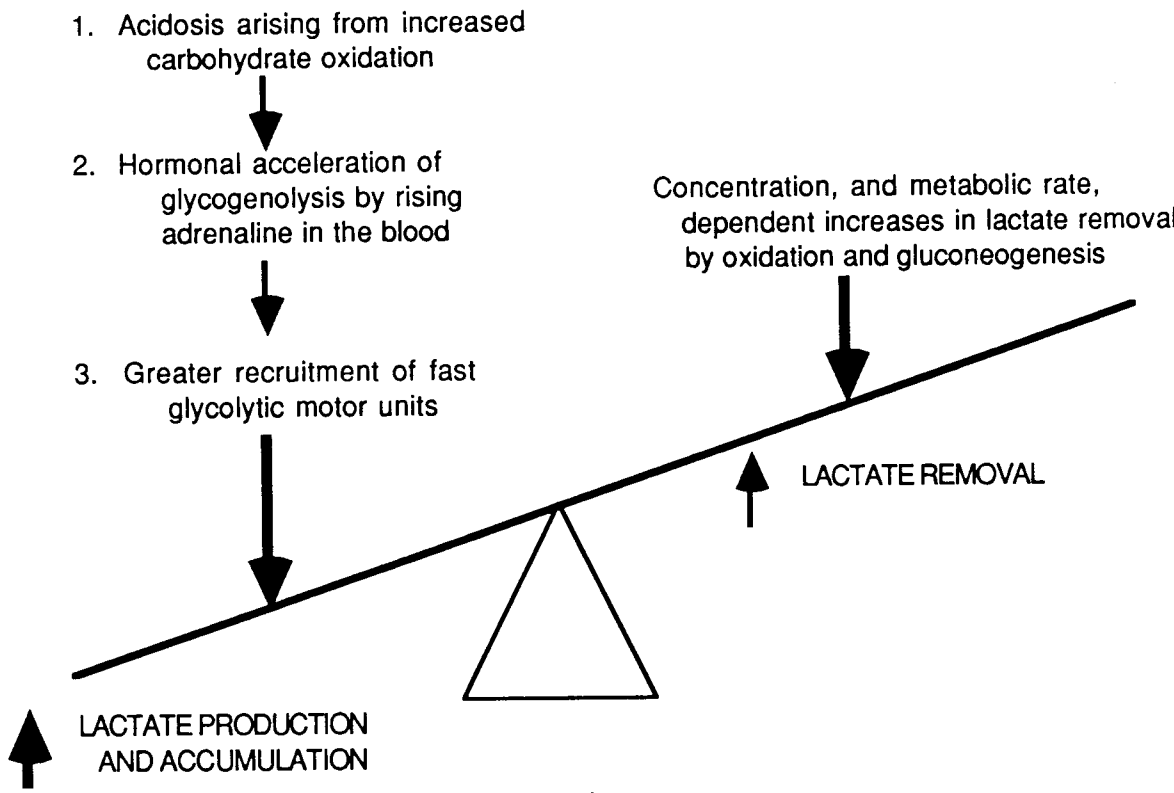


FIGURE 2.1 Summary of the factors likely responsible for the rise in lactate accumulation during progressive exercise.

CHAPTER 3

METHODS

Subject selection

Ten men aged 21 to 45 were recruited by advertising the research project in local sporting goods stores and fitness centers close to the University of Cape Town. Following an initial interview by telephone, each subject was interviewed by the investigator, at which time the purpose, scope, methods, benefits and health risks associated with the project were explained. Upon verbal agreement to participate in the study a time was determined for initial testing for each subject. Prior to undergoing the initial testing, each subject gave his written informed consent to participate in the study. All protocols were approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. Eight subjects completed the study, two having withdrawn from the project for personal reasons.

Training Programme

The 9 week training programme consisted of supervised exercise four days per week for approximately 45 minutes per session on stationary cycle ergometers, with approximately 1 hour of independent cycling on a 5^{th} day each week. Following the initial pre-training test sessions the initial training intensities were calculated from the 2 mM and 4 mM blood lactate concentrations achieved during the 20W $.min^{-1}$ incremental exercise test. The heart rates attained at these specific blood lactate concentrations were used to establish the initial training intensities.

Table 3.1 illustrates the initial training work rates for each subject. The subjects were required to exercise at an intensity no lower than that which elevated their blood lactate concentrations to 2 mM. The steady-state portion of the exercise session was conducted at an exercise intensity that was closer to the subject's 4 mM (84% VO_2max) than 2 mM lactate (60% VO_2max) concentration.

TABLE 3.1. PRE-TRAINING HEART RATES AT 2 AND 4 mM LACTATE CONCENTRATIONS

	Heart Rate			
Name	2 mM	%HRmax	4 mM	%HRmax
PS	140	80	151	88
DC	144	72	183	92
GM	121	65	160	86
MS	109	61	128	72
JVN	132	69	160	83
LV	118	61	140	73
RW	139	70	184	92
QVC	118	62	164	86

By the third week of the training programme, the supervised exercise consisted of: (i) an eight minute warm-up period at approximately 40% VO_2max; (ii) a 20-25 minute period of steady-state exercise at approximately 70% VO_2max; (iii) a 10-12 minute period of high

intensity interval-type exercise (30-120 sec exercise at maximal heart rate; heart rate was monitored by auscultation immediately upon completion of each interval), and (iv) a 2-5 minute cool-down period. Heart rate response to the exercise was adjusted on a day-to-day basis to maintain relative training intensity at constant levels.

VO_2max determination.

Subjects reported to the laboratory at least 6 hours after eating. Maximum oxygen consumption (VO_2max) and blood lactate accumulation were measured pre- and post-training during progressive exercise to exhaustion on an electrically braked cycle ergometer (Tunturi EL400, Tunturipyora, Piisparisti, Finland). In these tests, the initial work rate was 60 W and the work-jump increments were 20 W every minute. During the exercise, the subjects wore a nose-clip and inspired air from a Hans Rudolph 2700 (Vacumed, Ventura, CA) one-way valve connected to a Mijnhardt dry gas meter. Expired air was passed through a 15 L baffled mixing chamber and a condensation coil to Ametek N-22 M O_2 and CD-3 A CO_2 gas analyzers (Thermox Instruments, Pittsburgh, PA). Before every test, the gas meter was calibrated with a Hans Rudolph 5530 3 L syringe and the analyzers were set with air and a 4% CO_2: 16% O_2: 80% N_2 mixture. Instrument outputs were processed by an on-line IBM PC computer which calculated the average V_I, VO_2 and VCO_2 over each minute using conventional equations. Heart rates were recorded each minute from a RA, LA, V5 electrocardiogram by a Lohmeier ECG monitor (Lohmeier, West Germany)

Venous "arterialized" blood was continuously sampled from an indwelling Jelco 22 guage cannula in a left forearm vein connected to an Eyela Microtube pump (Rakakekai, Japan). Blood leaving the pump ($1ml.min^{-1}$) was deproteinized in 0.6 M ice-cold $HClO_4$ (2 ml),

cold-centrifuged at 500 x g for 15 min and stored at -20°C for later enzymatic assay (Gutmann, & Wahlefeld, 1974).

^{14}C - lactate infusion test.

The subjects were studied in the morning after a 12-14 hour fast and at least 48 hours after their last exercise session. The subjects were instructed to maintain a regular diet, and to record this for the last three days prior to the testing. This same diet (approximately 48% carbohydrate, 36% fat and 16% protein) was also followed for the 3 days prior to the subsequent testing following training. For L-[U-^{14}C] lactate infusion a 22 guage Jelco catheter was inserted into a left forearm vein. For "arterialized" venous blood sampling a 20 guage Jelco catheter was inserted in a right forearm vein and then covered with a heating pad. This latter catheter was kept patent with saline (Sabax NaCl 0.9%). Sterile (0.3 uCi.ml^{-1}) solutions of L-[U-^{14}C] lactate (169 mCi/mmol, Amersham Laboratories) in 0.9% NaCl were infused at a constant rate of approximately 10 uCi. hour^{-1} from an Auto Syringe model 5C pump (Travenol labs, Nookset, N. H.). Prior to use an aliquot of the labeled lactate was sent to the State Vaccine Institute to insure that the sample was sterile and pyrogen free.

For the first 45 minutes of the L-[U-^{14}C] lactate infusion, the subjects rested while steady-state blood lactate specific radioactivities were achieved (Gertz, et al., 1981). Following the withdrawal of two 1 ml blood samples at three minute intervals to confirm that blood lactate specific radioactivities were constant, the subjects then began progressive exercise tests to exhaustion on a Tunturi EL400 cycle ergometer. Again the initial work rate was 60 W but, in this case, the work-jump increment was 40 W every 6 min rather than 20 W per minute as for the VO_2max test. Exercise began within 2 minutes after the taking of the second resting

blood sample. Blood for the determination of plasma lactate and ^{14}C-lactate was sampled at three minute intervals, and blood for determination of plasma free fatty acid, glucose, insulin and glucagon concentrations was taken at 6 minute intervals.

During the 6 min work stages, the subjects were instructed to maintain a cadence of 80 rpm and VO_2, VCO_2 and V_I were measured during the last 3 minutes of each work stage using the system described above. In this period, $l.min^{-1}$ VO_2 values varied by less than 0.1 $l.min^{-1}$ indicating that metabolic rates were relatively stable. Heart rates were also constant over the final 3 min of each exercise stage. An aliquot of expired CO_2 was collected during the last 90 seconds of each work stage for determination of $^{14}CO_2$ production.

Blood L-(U-^{14}C)-lactate and (U-^{14}C)-glucose measurements

Samples of 1 ml of blood collected during the infusion of L-[U-^{14}C]-lactate, were kept on ice in Evergreen test-tubes (Evergreen Scientific, Los Angeles) and deproteinized by adding 0.1 ml of 50% v/v $HClO_4$. Deproteinized samples were then buffered by slowly adding 0.6 ml of 4M K_2CO_3 in Pipes buffer Piperazin - 1,4 - bis (Merck, Art. 10220), and then briefly mixed. The pH was then adjusted to 6.8 - 7.2 with the aid of a Schotte Ger'a'te CG820 pH meter (Hofheim, West Germany). The samples were cold- centrifuged at 4000 rpm for 10 minutes at 400 x g and the supernatant was removed and placed on ice. Distilled H_2O (0.4 ml) was added to the sample and mixed briefly. The sample was once again centrifuged as described above, and the supernatant added to that previously removed.

Combined supernatants were then passed through 1 cm x 4 cm anion exchange resin columns of Dowex-1-C1 (Chloride form [60267-37-0]; 2% cross-linked, dry mesh 50-100. Sigma Chemical Co., St.Louis, Missouri). Glucose was eluted into 20 ml Kimble

borosilicate glass vials (Owens-Illinois, Toledo, Ohio) by passing 5 ml of distilled H_2O through the column. Lactate was eluted into borosilicate glass vials by passing 5 ml of 0.2M $CaCl_2$ through the column. The samples were then placed in an oven at 70°C for approximately 20 hours to reduce the volume to less than 1 ml.

10 ml of liquid scintillation cocktail (Beckman Ready Gel, [P/N158728], Beckman Instruments Inc., Fullerton, California) was then added to each evaporated sample. ^{14}C disintegrations per minute (dpm) were determined by counting each sample for 10 minutes in a Packard Tri-Carb 4640 liquid scintillation counter (Packard Instrument Co., Downers Grove, Illinois).

The rate of lactate appearance (Ra) and the rate of lactate disappearance (Rd) from the lactate space were calculated using the nonsteady-state equations of Steele (1959), shown below:

(1) $$Ra = [F - (V \times \overline{LA} \times \Delta SA/\Delta t)]/\overline{SA}$$

(2) $$Rd = Ra - (V \times \Delta LA/\Delta t)$$

In these equations, F is the $dpm.min^{-1}.kg^{-1}$ infusion rate (determined for each subject), V is the predicted 100 $ml.kg^{-1}$ non steady-state lactate distribution volume (Stanley, et al., 1985), $\overline{LA}$ is the $umol.ml^{-1}$ mean lactate concentration in consecutive samples, $\Delta SA/\Delta t$ is the $dpm.umol^{-1}.min^{-1}$ change in lactate specific radioactivity, $\overline{SA}$ is the mean $dpm.umol^{-1}$ lactate specific radioactivity in successive samples and $\Delta La/\Delta t$ is the $umol.ml^{-1}.min^{-1}$ change in lactate concentration. Units of Ra and Rd are $umol.min^{-1}.kg^{-1}$ body mass.

In addition to calculating lactate Ra and Rd values, we also calculated $ml.kg^{-1}.min^{-1}$ lactate metabolic clearance rates (MCR). MCR data were obtained by dividing $umol.min^{-1}.kg^{-1}$ Rd values by corresponding $umol.ml^{-1}$ LA concentrations.

$^{14}CO_2$ measurements

Aliquots of expired CO_2 for $^{14}CO_2$ measurements were collected from an extension line attached to the outlet port of a CO_2 analyzer (Ametek CD-3 A, Thermox Instruments, Pittsburgh). Gas leaving the analyzer was bubbled through a mixture of 1 ml hyamine hydroxide (1N solution in methanol, Packard Instrument Co., Downers Grove, Illinois), 1ml ethanol (Merck), and 2 to 3 drops of 1% phenolthalein (Lab. and Scientific Equipment Co., Cape Town) until the colour changed from pink to clear. At this point 1 mmol of CO_2 had been collected (Scherrer, Haldimann, Kupfer, Reubi, & Bircher, 1978). Liquid scintillation cocktail was added for radioactivity counting as described previously.

Estimates of lactate oxidation

Initially (because 40-50% of the ^{14}C-label was in blood glucose), attempts were made to report the oxidative decarboxylation of ^{14}C-lactate plus ^{14}C-glucose from lactate in terms of the expired $V^{14}CO_2$ $dpm.min^{-1}$ as follows (Eq. 3) :

$$(3) \qquad V^{14}CO_2\ dpm.min^{-1} = VCO_2\ mmol.min^{-1} \times {}^{14}CO_2\ dpm.mmol^{-1}$$

However, the interpretation of the higher $V^{14}CO_2$ values after training was complicated by the initially lower lactate Ra and Rd values in the trained subjects. Decreased lactate turnover reduced blood lactate concentrations, increased ^{14}C-lactate specific radioactivities

and slowed the transfer of ^{14}C-label from lactate to glucose via gluconeogenesis. All of these changes were expected to have influenced the rates of $^{14}CO_2$ release. Corrections therefore had to be made for the differences in circulating ^{14}C-levels before and after training. One approach was to calculate the $l.min^{-1}$ ^{14}C-clearance by oxidation as follows (Eq 4):

(4) $$^{14}C\text{-clearance } l.min^{-1} = V^{14}CO_2 \text{ dpm.min}^{-1} / {}^{14}C_{total} \text{ dpm.min}^{-1}$$

Here, $^{14}C_{total}$ $dpm.min^{-1}$ were calculated by multiplying the ^{14}C-lactate and ^{14}C-glucose $dpm.mmol^{-1}$ specific radioactivities by their corresponding $mmol.l^{-1}$ concentrations and summing the products.

Another approach was to attempt to correct for the $V^{14}CO_2$ from ^{14}C-glucose oxidation and tentatively estimate the rates of ^{14}C-lactate oxidation (lac R_{ox}) in $umol.min^{-1}.kg^{-1}$. With 40-50% of the ^{14}C-label in blood glucose, such corrections were needed to prevent an over-estimation of lac R_{ox}.

Corrections for ^{14}C-glucose oxidation were made using Stanley et al.'s (1988) data on glucose and lactate turnover in exercising humans. From (i) their glucose and lactate Rd values of 3.3 and 3.5 $mg.min^{-1}.kg^{-1}$ and (ii) their finding that the conversions of glucose to lactate and lactate to glucose are both around 25% of the total Rd's, we calculated that the $umol.min^{-1}.kg^{-1}$ lactate to glucose oxidation ratio is approximately 2 : 1 during moderate exercise, where blood lactate concentrations remain at around 1 $mmol.l^{-1}$.

Accordingly, the rates of ^{14}C-lactate oxidation (Lac R_{ox}) in $umol.min^{-1}.kg^{-1}$ were estimated as follows (Eq. 5):

(5) $\text{lac } R_{ox} = [SA_{CO2} \cdot (SA_{lac} + 0.5\ SA_{glu})^{-1}] \times VCO_2 \cdot 3^{-1}$

where SA_{CO2} is the specific radioactivity of expired $^{14}CO_2$ in dpm. $mmol^{-1}$, SA_{lac} and SA_{glu} are the 2 : 1 ratio of the blood ^{14}C-lactate and ^{14}C-glucose specific radioactivities in dpm. $mmol^{-1}$, VCO_2 is the CO_2 production in $umol.min^{-1}.\ kg^{-1}$, and 3^{-1} is the correction for 3 molecules of CO_2 being produced from the oxidation of each lactate molecule.

Since the rising blood lactate concentrations during progressive exercise would be expected to increase the lactate to glucose oxidation ratio, a maximum $umol.min^{-1}.\ kg^{-1}$ lac R_{ox} was also calculated by not correcting for the $V^{14}CO_2$ from ^{14}C-glucose oxidation (Eq. 6):

(6) $\text{lac } R_{ox} = (SA_{CO2} \cdot SA_{lac}^{-1}) \times VCO_2 \cdot 3^{-1}$

Differences between corrected and uncorrected Lac R_{ox} values were used to see how the potential rise in the lactate to glucose oxidation ratio with increasing blood lactate concentrations might influence the results. Whereas the circulating lactate concentration in Stanley et al's subjects remained at around 1 $mmol.l^{-1}$ (Stanley, et al., 1988), in these subjects, it progressively increased to around 6 $mmol.l^{-1}$ (Figure 4.2 B, Chapter 4).

Results presented are the corrected $umol.min^{-1}.kg^{-1}$ Lac R_{ox} values. How an over-correction may affect the findings is described in the section describing lactate removal by oxidation and gluconeogenesis during exercise.

Estimates of lactate removal via gluconeogenesis

As with the estimates of lactate oxidation, measurements of lactate removal via gluconeogenesis (lac R_{glu}) also had to be corrected for the higher ^{14}C-blood lactate specific radioactivities after training. First, this was achieved by calculating the percentage of glucose labeled by ^{14}C-lactate as follows (Eq. 7):

(7) $$\% \text{ glu} = [(\text{glu dpm. mmol}^{-1} / (\text{lac dpm.mmol}^{-1})] \times 100$$

Then, lac R_{glu} in umol.min^{-1}.kg^{-1} was estimated from the difference between the corrected umol.min^{-1}.kg^{-1} lac R_{ox} values from Eq. 5 and the overall umol.min^{-1}.kg^{-1} rates of lactate disappearance (Rd; Eq. 2), using Eq. 8:

(8) $$\text{lac } R_{glu} = \text{lac Rd umol.min}^{-1}\text{.kg}^{-1} - \text{lac } R_{ox} \text{ umol.min}^{-1}\text{.kg}^{-1}$$

Blood substrates and hormones

Determinations of blood lactate, glucose, glucagon, insulin and plasma free fatty acid concentrations were carried out in duplicate according to the procedures outlined in appendix A. Blood lactate concentrations were measured at rest and at three minute intervals during the infusion tests. Glucose, free fatty acid, glucagon and insulin concentrations were analyzed at rest and at six minute intervals during the infusions.

Anthropometric assessment.

The height of the subjects (cm) was determined prior to the first exercise test and their weight (kg) was measured prior to all exercise tests. Both before and after the training program, the body compositions of the subjects were determined according to standardized anthropometric procedures. Briefly, subcutaneous fatfolds were determined in triplicate using Holtain skinfold calipers (England) on the right biceps, triceps, subscapula and suprailiac sites as described by McArdle et al. (1991). With the exception of the subscapula and suprailiac folds which were measured in the oblique plane, all fatfolds were measured in the vertical plane.

The mean of the three fatfold measurements at each site was calculated and the equivalent fat content was determined as a percentage of body weight according to the method of Durnin and Womersley (1974). Fat weight (kg) was calculated as percent fat x body weight. Lean body weight (kg) was calculated as body weight - fat weight. Body composition results are given in table 3.2.

TABLE 3.2. BODY COMPOSITION BEFORE AND AFTER TRAINING

Variable	Before training	After training
Height (m)	1.78 ± 0.05	same
Mass (kg)	87.9 ± 5.6	86.5 ± 5.5
% fat	24.5 ± 2.2	23.4 ± 2.3
Fat mass (kg)	22.1 ± 3.0	20.8 ± 3.0
Lean mass (kg)	65.7 ± 2.8	65.7 ± 2.8

Values are means ± S.E. for 8 subjects. No statistically significant differences were seen after training.

Muscle Biopsy Procedure

Prior to, and at the conclusion of the L-[U-^{14}C] lactate infusion experiments, a muscle biopsy was taken from the right vastus lateralis muscle of each subject for determination of muscle fibre type, muscle fibre size and muscle glycogen content. The method used to perform the vastus lateralis biopsy was that of Bergstrom (1962) as modified by Evans et al. (1982).

The needle biopsy set was autoclaved before all biopsy procedures. Prior to the biopsy a plastic catheter adaptor (Tomac, 21627-010) was inserted into the central channel of the biopsy needle (Stille Werner). An extension tube was used to connect the adaptor to a 50 ml disposable syringe according to the method of Evans et al. (1982). The area above the belly of the vastus lateralis muscle (approximately 15 to 20 cm above the knee) was shaved, cleaned with betadine antiseptic solution, and the skin and outer layer of the subcutaneous tissue were anaesthetized locally with 2 ml of Lignocaine (Labethica Pty. Ltd., Bethlehem, South Africa). When the site was no longer sensitive, a sterile surgical blade was used to make an incision of approximately 1 cm in length through the skin, subcutaneous tissue and the fascia overlying the muscle. The tip of the biopsy needle with inner cylinder closed was rapidly introduced approximately 2.5 cm deep into the muscle. The inner cylinder was then retracted a few centimeters while the outer needle remained in place. An assistant pulled back on the plunger of the 5 ml syringe, causing a suction force which pulled the muscle tissue surrounding the window of the hollow needle into the central opening of the needle. While maintaining suction, the central cylinder was then pushed in sharply, cutting off the small piece of muscle which had protruded into the central bore of the needle. As soon as the cutting blade was closed, the suction was immediately released.

The whole needle was then rapidly removed from the muscle, a section of the biopsied tissue was dissected out for fibre typing, and the remaining tissue rapidly immersed in liquid nitrogen in order to freeze the biopsy sample. A sterile forceps and stylet were then used to extract the frozen piece of tissue from the needle. This tissue was then place in an Eppendorf tube and kept in a freezer at -80° C until analyzed. Immediately following the removal of the needle from the biopsy site, a dressing and compression was applied to the wound until bleeding stopped. The incision was then closed with Clearon skin closures

(Ethicon Inc., New Jersey, U.S.A.) and an Opsite wound dressing (Smith and Nephew, Natal, South Africa) placed over the biopsy site.

Determination of muscle fibre type, size and glycogen content was carried out according to the procedures outlined in appendix A. The muscle fibre characteristics of the subjects are summarized in table 3.3.

TABLE 3.3. MUSCLE FIBRE TYPE AND DISTRIBUTION BEFORE AND AFTER TRAINING

	Before training		After training	
	Type 1 fibre	Type 2 fibre	Type 1 fibre	Type 2 fibre
Diameter (μ)	46.4 ± 2.0	52.7 ± 1.8	51.2 ± 2.8*	59.2 ± 2.2**
Percentage (%)	46 ± 4	54 ± 4	47 ± 4	53 ± 4

Values are means ± S.E. for 8 subjects. Statistically significant differences after training are denoted by * $p < 0.05$, and ** $p < 0.01$. Type 1 and 2 fibre diameters increased 9 and 11% respectively after training.

Curve-fitting analyses of metabolic responses and lactate accumulation

Rises in VO_2 and heart rate with increasing work rate were investigated with the Cricket Graph curve-fitting program (Cricket Software, Malvern, Pennsylvania). Inter-relationships between the rises in V_I and VCO_2 with increasing VO_2, and the rise in blood $[La^-]$ with increasing VO_2 were investigated with the ISI Graph Pad program (Institute for

Scientific Information, Philadelphia, Pennsylvania). Plots of lactate concentration [La^-] vs. O_2 consumption were analyzed by a formal optimization program. In this program, the differences between the observed accumulation rates of La^- and those computed from the trial La^- = A. exp. [B. VO_2] + C rate equations were minimized by non-linear regression. Curves from these equations were then compared by converting the differential of the exponential rate equation, dLa/dVO_2 = A. B. exp. [B. VO_2], to a natural logarithmic form, ln dLa/dVO_2 = [ln A. B.]. B. VO_2, and re-arranging it to give (Eq. 9):

(9) VO_2 = [ln [[dLa/dVO_2]/A. B.]]/B

With this expression, l. min^{-1} VO_2's at dLa/dVO_2 slopes of 1, 2 and 3 were determined. Then, accelerations of ventilation volume (dV_I/dVO_2) at the 1, 2, and 3 "lactate slopes" were calculated by introducing the lactate slope VO_2's into the differential of the V_I = A. exp. [B. VO_2] + C rate equation (Eq. 10):

(10) dV_I/dVO_2 = A. B. exp.[B. VO_2]

Then, the individual 1, 2, and 3 lactate slope VO_2's were introduced.

Statistical Analyses

Effects of training on lactate production and removal during progressive exercise

All subjects were able to complete at least 30 min of exercise during the U-^{14}C-lactate infusion before and after training. Therefore, a two-way analyses of variance for repeated measures was used to assess the statistical significances ($p< 0.05$) of the differences in lactate concentration, lactate Ra, Rd and MCR, only up to 30 min of exercise before and after training. When significant interactions were found these were examined further by tests of simple main effects. Peak values for lactate concentration, lactate Ra, Rd and MCR before and after training were compared using paired Student's t tests.

Effects of training on lactate removal by oxidation and gluconeogenesis during exercise

As above, a two-way analyses of variance for repeated measures was used to assess the statistical significances ($p< 0.05$) of the differences in lac R_{ox}, lac R_{glu}, % glucose labeled and overall CHO oxidation, only up to 30 min of exercise before and after training. When significant interactions were found, these were examined further by tests of simple main effects. Blood metabolite and hormone concentrations, and changes in muscle glycogen contents before and after training, were compared using paired Student's t tests.

Effects of training on respiratory responses during progressive exercise

Since the exercise tests after training resulted in a longer time to exhaustion, statistical significance ($p < 0.05$) was determined as follows; a two-way analyses of variance was used to compare HR, VO_2, V_I and VCO_2 responses in the 20W. min^{-1} ramp test up to a work rate of 260 W, and in the 40W. 6 min^{-1} ramp test up to a work rate of 220 W. Comparisons between the two ramp slopes were made up to a work rate of 220W. When significant interactions were found, these were examined further by tests of simple main effects. The R^2 values of the non-linear (exponential) least-squares analyses, and linear regression of the individual VO_2 and HR curves with increasing work rate, VO_2's at dLa/dVO_2 slopes, and dV_I/dVO_2 values at equivalent rates of blood lactate accumulation, were assessed by one-way analysis of variance.

CHAPTER 4

EFFECTS OF TRAINING ON LACTATE PRODUCTION AND REMOVAL DURING PROGRESSIVE EXERCISE

INTRODUCTION

Although it is well known that endurance training attenuates blood lactate accumulation during exercise, the mechanisms responsible remain to be defined. More specifically, why does exercise at a given absolute workrate which is submaximal in the pre-trained state, and which accordingly represents a lower percentage of the post-training VO_2max, elicit reduced blood lactate concentrations during submaximal exercise after training? One explanation is that training decreases lactate production from carbohydrate utilization by increasing skeletal muscle mitochondrial density (Holloszy, 1967; Holloszy, et al., 1984; Morgan, Cobb, Short, Ross, & Gunn, 1971) and improving fatty acid oxidation (Hickson, Rennie, Conlee, Winder, & Holloszy, 1977; Rennie, Winder, & Holloszy, 1976). Endurance-trained athletes also rely to a lesser extent on muscle glycogen (Karlsson, Nordesjo, & Saltin, 1974; Saltin, 1969) and, maintain blood lactate concentrations near resting levels during prolonged high-intensity exercise (Costill, Thomason, & Roberts, 1973b).

The lower blood lactate concentrations seen after endurance training are therefore likely partly due to changes in substrate use. Increases in muscle mitochondrial mass and the activity of mitochondrial enzymes, allowing for greater ATP production via oxidative processes, would decrease glycolytic flux and thus lactate formation. The improvement in fat oxidation as a result of training would decrease the requirement for ATP formation via glycolysis, and reduce the rate of production of lactate.

Another proposal for the diminished blood lactate accumulation during exercise after training, is that it is the rate of lactate clearance, rather than the rate of lactate formation, that is altered by endurance training. In support of this argument are the radioactive tracer studies of Donovan and Brooks (1983) in rats, and of Stanley et al. (1985) in a competitive runner and a recreational swimmer. A more recent investigation by Donovan and Pagliassotti (1990a) confirmed these findings by showing higher rates of lactate removal in endurance trained rats.

Here, we show that the reduced blood lactate concentrations during submaximal exercise after training in humans, are the combined result of a decreased rate of lactate appearance and an improvement in the rate of lactate clearance.

RESULTS

Physical performance

Physiological data for the eight subjects before and after 9 weeks of training are given in Table 4.1. Prior to training 5 subjects completed 30 min (220 W) of the 40 W every 6 min graded exercise test and three subjects completed 36 min (260 W). Following the training program 7 subjects were able to continue exercising for 42 minutes (300 W), and 1 subject stopped after 39 minutes (300 W). In the pre-training 40 W every 6 min exercise test, peak VO_2 was 2.27 ± 0.07 l.min^{-1} and, after training, this value was increased to 2.60 ± 0.07 l. min^{-1} ($p < 0.05$).

TABLE 4.1. THE EFFECTS OF TRAINING ON SELECTED CHARACTERISTICS OF THE SUBJECTS

	Pre-training	Post-training
Mass (kg)	88 ± 5.0	87 ± 5.0
% Body fat (a)	24.5 ± 2.2	23.4 ± 2.3
VO_2max ($l.min^{-1}$) (b)	2.59 ± 0.1	3.45 ± 0.2*
Maximum work rate (Watts)	220 ± 15.1	300 ± 0.5*
HR_{rest}	80 ± 1.0	64 ± 2.0*
HR_{max}	185 ± 4.0	189 ± 4.0

Values are the means and S.E.'s of the subjects' data (* $p < 0.05$, a significant training effect)

(a) % body fat was calculated from biceps, triceps, subscapular and suprailiac skin fold measurements using the equation of Durnin and Wolmersley (1974).

(b) VO_2 max and maximum heart rate values are from the 20 W per min exercise tests, and maximum workrate values are from the 40 W every 6 min exercise tests.

Blood lactate accumulation

Changes in blood lactate specific activity (LSA) before and after training are shown in Figure 4.1 A. Blood LSA was not altered by training at rest, but was higher from 6 to 30 min of exercise after training ($p < 0.01$). Rates of blood lactate turnover and accumulation with increasing VO_2 are shown in Figures 4.1 B, 4.2 A and 4.2 B. Before training, lactate

concentrations climbed exponentially from 1.19 ± 1.0 to 7.44 ± 2.5 mmol.l^{-1} as VO_2 rose linearly from 0.88 ± 0.04 to 2.27 ± 0.07 l.min^{-1}. After training, similar degrees of peak blood lactate concentration were only seen at a VO_2 of 2.6 ± 0.07 l.min^{-1} (Figure 4.1 B).

Rates of blood lactate appearance

Delays in blood lactate accumulation after training were partly due to a reduced rate of lactate appearance. Over the lower 1.10 to 2.23 l.min^{-1} VO_2 range, rates of lactate appearance (Ra) were significantly reduced by training (Figure 4.2 A; $p < 0.01$). At the peak VO_2's however, lactate Ra values were similar. Both before and after training, subjects terminated exercise, when rates of lactate appearance rose to 214.5 ± 28 and 243.8 ± 12 umol.min^{-1}.kg^{-1} respectively (Figure 4.2 A; $p > 0.05$). When expressed relative to VO_2max, lactate Ra was only lower at work rates up to 60% VO_2max after training (Table 4.2; $p < 0.01$). At work rates higher than 60% VO_2max, lactate Ra's became similar before and after training.

Rates of blood lactate disappearance

A decrease similar to that of lactate Ra was observed for lactate Rd over the lower 1.10 to 2.23 l.min^{-1} VO_2 range (Figure 4.2 B; $p < 0.01$). In contrast to lactate Ra, the peak rates of blood lactate disappearance at test termination were higher following training. Despite the lower circulating lactate concentrations after training (Figure 4.1 B), the maximum lactate Rd at exhaustion was increased from 177.1 ± 25 to 223.7 ± 11 umol.min^{-1}.kg^{-1} (Figure 4.2 B; $p< 0.05$). When expressed relative to VO_2max, lactate Rd was only higher at work rates beyond 60% VO_2max after training (Table 4.2; $p < 0.05$). At work rates below 60% VO_2max, lactate Rd's were similar before and after training.

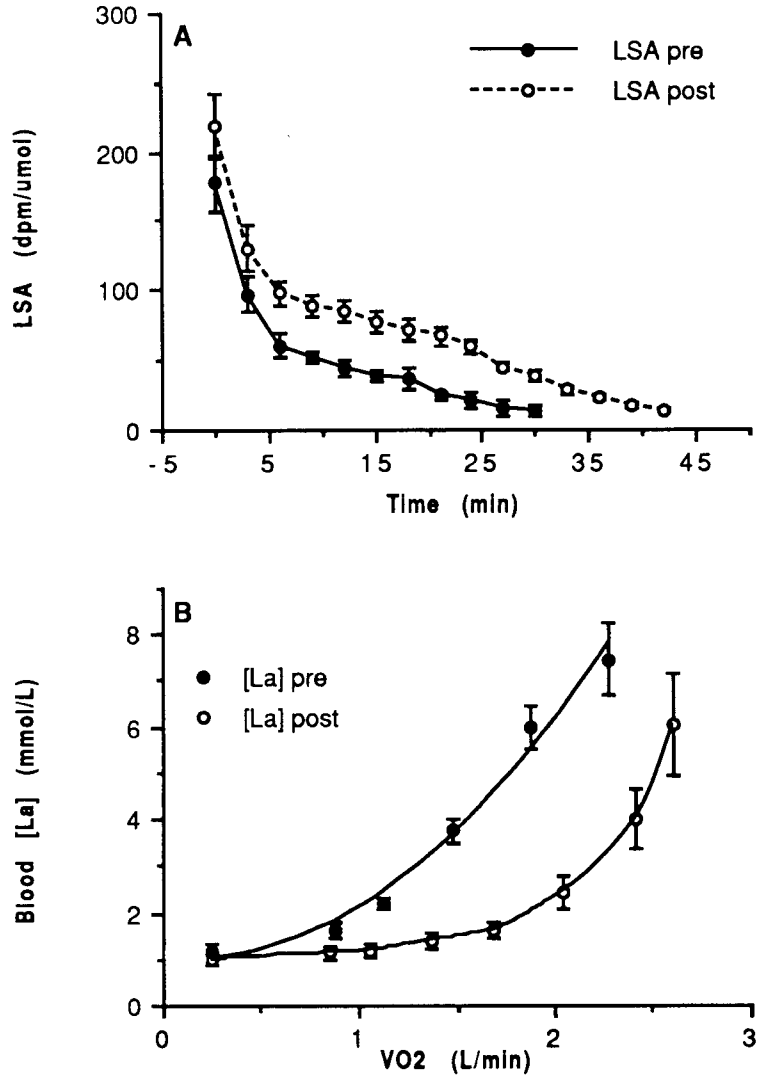


FIGURE 4.1. The effect of increasing work rate before and after training on lactate specific activity (LSA; panel A) and blood lactate accumulation (panel B). Blood lactate accumulation data before and after training were fitted with $y = A.e^{Bx} + C$ equations ($R^2 = 0.993$ before training, and $R^2 = 0.998$ after training). LSA was higher and lactate accumulation lower at work rates eliciting VO_2's of 0.85 to 2.27 $l.min^{-1}$ after training ($p < 0.01$).

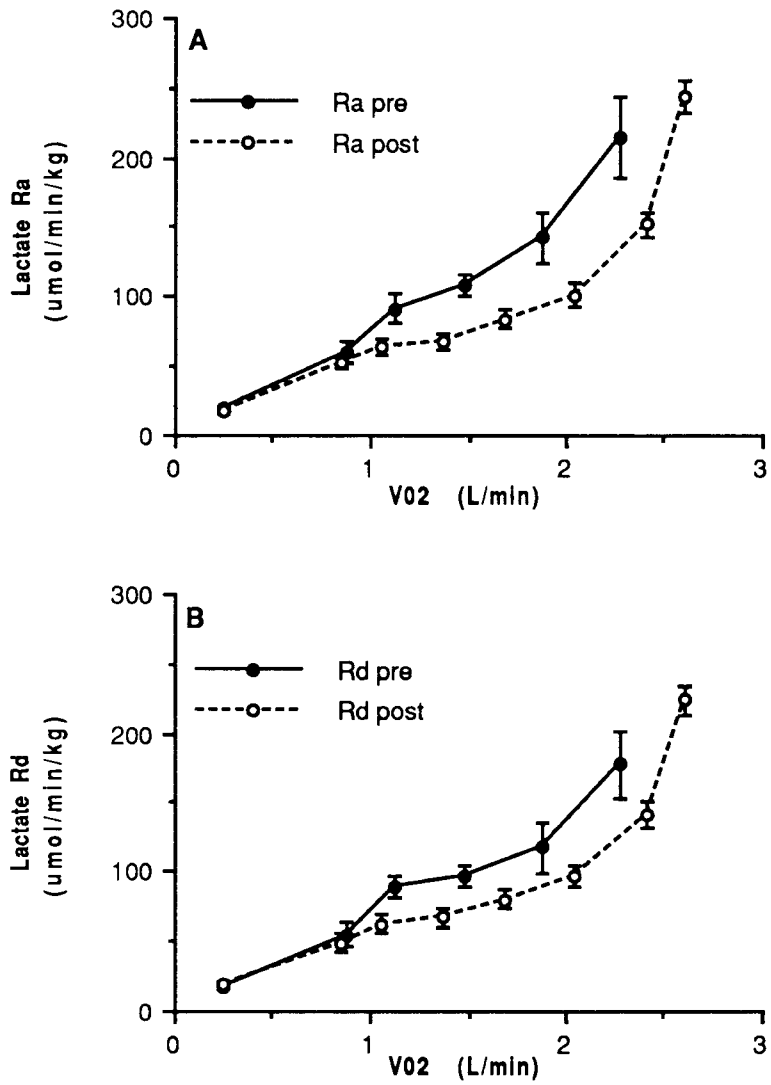


FIGURE 4.2. Mean ± S.E. blood lactate Ra's (panel A), and lactate Rd's (panel B) during a progressive exercise test in eight sedentary adult males before and after nine-weeks of training on a bicycle ergometer. Lactate Ra's and Rd's were significantly lower at VO_2's of 1.06 to 2.27 $l.min^{-1}$ after training ($p < 0.01$). Peak lactate Ra's were similar, but peak lactate Rd was higher after training ($p < 0.01$).

The reduced lactate Ra's and Rd's need to be considered in relation to the decrease in overall carbohydrate (CHO) oxidation after training. Following the training programme, respiratory exchange ratio (RER) values at, for example, a VO_2 of 2.0 $l.min^{-1}$, were decreased from 0.96 ± 0.02 to 0.90 ± 0.01 (Figure 4.4; $p < 0.01$).

Rates of blood lactate metabolic clearance

Lactate metabolic clearance rates (MCR) at higher VO_2's were also improved by training (Figure 4 .3 A). After training, MCR remained elevated at VO_2's of 1.48 to 2.27 $l.min^{-1}$ ($p < 0.01$), and the extent to which it fell at test termination was limited to 40.2 ± 3.5 as opposed to 31.3 ± 3.6 $ml.min^{-1}.kg^{-1}$ ($p< 0.01$). The slopes of the lines in Figure 4.3 B illustrate the differences in lactate MCR as a result of training. Here, lactate clearance was increased at blood [La]'s higher than 4 $mmol.l^{-1}$ after training (Figure 4.3 B; $p < 0.05$). In addition, the lactate MCR was significantly higher at work rates up to 75% VO_2max after training (Table 4.2; $p < 0.01$).

TABLE 4.2: SUMMARY OF SUBMAXIMAL AND PEAK LACTATE AND METABOLIC DATA

	Before-training			After-training		
Percentage VO_2	40%	60%	Peak	40%	60%	Peak
VO_2 ($l.min^{-1}$)	1.04±0.1	1.55±0.1	2.27±0.1	1.38±0.1**	2.07±0.1**	2.60±0.1*
[Lactate] ($mmol.l^{-1}$)	3.48±0.2	4.67±0.3	7.44±0.8	2.23±0.1**	2.89±0.1**	6.03±1.1
Lactate Ra (umol/min/kg)	103±5	133±8	215±28	90±4**	117±6	244±12
Lactate Rd (umol/min/kg)	91±4	113±6	177±25	94±4	123±6	225±11*
Lactate MCR (ml/min/kg)	26±0.4	24±0.4	31±4	42±0.1**	42±0.1**	40±3**

Values are means ± SE for 8 subjects during the infusion test. Statistically significant differences after training are denoted by * $p< 0.05$ and ** $p < 0.01$.

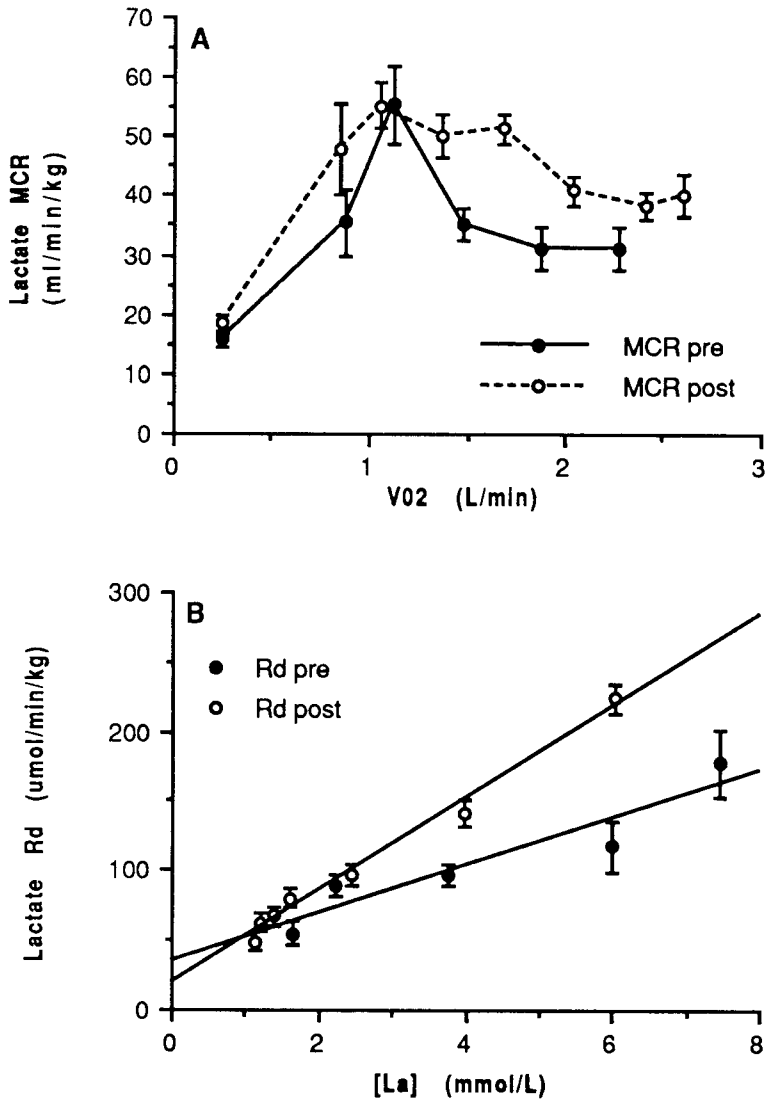


FIGURE 4.3. Mean changes ± S.E. in lactate metabolic clearance rate before and after training (panel A), and lactate Rd expressed as a function of increasing lactate concentration (panel B). Lactate MCR was elevated at VO_2's of 1.48 to 2.27 $l.min^{-1}$ after training ($p < 0.01$). Lactate Rd increased at blood lactate concentrations higher than 4 $mmol.l^{-1}$ after training ($p < 0.01$).

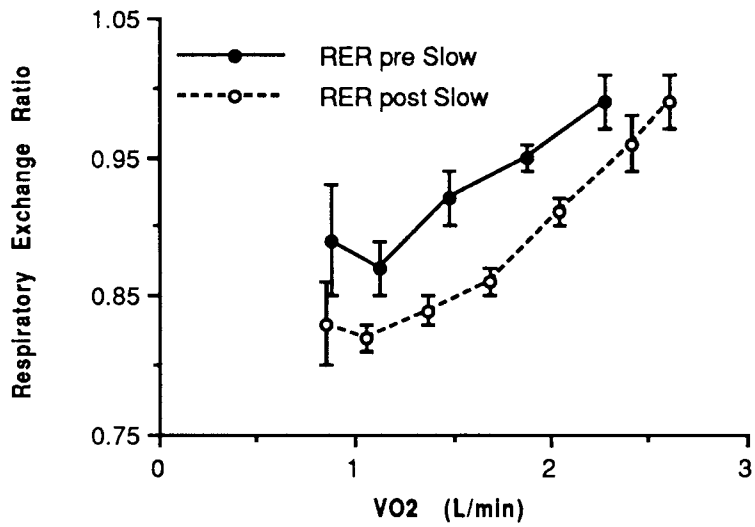


FIGURE 4.4. Mean ± S.E. changes in the respiratory exchange ratio (RER) before and after training. RER was lower at VO_2's of 1.06 to 2.27 $l.min^{-1}$ after training ($p < 0.01$).

Discussion of Results on Lactate Production and Removal

To our knowledge, this is the first longitudinal investigation into the effects of endurance training on lactate appearance and clearance during progressive exercise in humans. Previous studies on the effects of endurance training on blood lactate turnover at increasing work rates have been conducted in rats (Donovan & Brooks, 1983).

From the rat experiments, it was concluded that the reduction in blood lactate concentration during progressive exercise after training is more a result of an improved lactate metabolic clearance than of a decreased lactate appearance. A similar observation was made in a competitive runner and a recreational swimmer (Stanley, et al., 1985). In contrast to these findings, in our previously sedentary human subjects, we found that the lower circulating lactate concentrations during incremental exercise after training (Figure 4.1 B) were mainly due to a reduced lactate rate of appearance at the lower work rates (Figure 4.2 A; $VO_2 <$ 2.27 $l.min^{-1}$). In addition, part of the lower blood lactate concentrations seen after training, were due to the metabolic clearance of lactate being improved at the higher work rates (Figure 4.3 A; $VO_2 > 1.48$ $l.min^{-1}$).

Part of the improvement in metabolic clearance after training was due to the lactate Rd values being divided by lower corresponding blood lactate concentrations (Figure 4.3 B). The remainder was a consequence of an enhanced ability to remove high blood lactate concentrations [La] during exercise (Figure 4.3 A). After training, Rd values at blood [La]'s in excess of 4 mmol. l^{-1} were significantly increased (Figure 4.3 B; $p < 0.01$). This concentration dependency for lactate removal is consistent with the presence of a facilitated lactate transport mechanism (Roth, et al., 1990a).

Therefore, the diminished lactate concentration seen during exercise at equivalent metabolic rates, was the consequence of a combined process after training. During the initial stages of graded exercise when work rates were low, decreased lactate production likely caused a reduced lactate Ra as a result of mass action (Connett, Honig, Gayeski, & Brooks, 1990). During exercise at these lower work rates, lactate clearance was not improved by training. During the later stages of graded exercise, when work rates were high and lactate Ra's became similar, lactate accumulation remained low due to a sustained higher rate of lactate clearance after training. The high rate of lactate clearance at high work rates has also been seen in trained rats (Donovan & Brooks, 1983).

The large lactate Ra of around 20 $mg.min^{-1}.kg^{-1}$ in both the trained and untrained conditions during heavy exercise, is likely 4-5 times greater than the glucose flux (4-5 $mg.min^{-1}.kg^{-1}$). The contribution of this formed lactate as a fuel and gluconeogenic substrate, via the lactate shuttle (Brooks, 1986a), is likely to be significant in whole-body carbohydrate metabolism (Donovan & Brooks, 1983; Stanley, et al., 1988).

It needs however to be emphasized that the decreased rates of lactate appearance, after endurance training, have to be regarded as more directional than absolute. As demonstrated by Stanley et al. (1985), calculated lactate Ra and Rd values are greatly influenced by the non steady-state lactate distribution volume, and this volume is not known in exercising humans. It is likely that during prolonged submaximal steady-state exercise, the lactate distribution volume is larger than during short duration incremental exercise.

The use of a 100 ml. kg^{-1} non steady-state lactate distribution volume in calculations of human lactate turnover during exercise (Mazzeo, Brooks, & Schoeller, 1982; Mazzeo, et al., 1986; Stanley, et al., 1985; Stanley, Gertz, Wisneski, Neese, & Brooks, 1984) is based

on experiments in which glucose and urea were infused at different rates into dogs. In these studies, the least errors between known and calculated blood glucose and urea Ra changes were given by distribution volumes of 40 and 100 ml. kg^{-1} respectively (Allsop, Wolfe, & Burke, 1978; Wolfe, 1981). Whether a canine urea distribution volume can be applied to human lactate turnover during exercise, however, is uncertain. By selecting a 100 ml. kg^{-1} distribution volume, rather than a 40 or 50 ml. kg^{-1} distribution volume, it may be that lactate Ra and Rd values are under-estimated.

More to the point is the question of how endurance training affects lactate distribution. It is unknown whether training has any effect on the compartmentation of lactate, or whether training has any effect on the plasma membrane lactate carrier. A recent study by Roth and Brooks (1990a) using isolated non-metabolizing rat skeletal muscle sarcolemmal vesicles, identified a membrane-bound lactate transporter. Although the effects of training were not determined, these authors suggested a potentially important role for the lactate transporter as a bidirectional regulator of lactate flux across skeletal muscle.

Evidence from a recent investigation suggests, at least under resting conditions, that the lactate carrier is unlikely to limit lactate influx and efflux. In that investigation, Wolfe et al. (1988) demonstrated a rapid equilibration of ^{14}C-label between infused lactate and pyruvate in resting anaesthetized dogs. However, we do not agree with their conclusion that the methods used in our, and other similar tracer studies, more accurately reflect pyruvate than lactate kinetics. Lehman and Brooks (1990) have drawn attention to tracer studies in animals and humans which show the tracer measured lactate oxidation rate to be much less than the overall CHO oxidation (which would be equivalent if lactate and pyruvate specific activities were equivalent in vivo). In addition, tracer and non-tracer estimates of lactate Ra in resting men both provide values of around 1.2 $mg.min^{-1}.kg^{-1}$.

We suggest that the increased vascularization of the trained human muscles, as a consequence of training, may help to limit the lag between lactate efflux and its appearance in the blood. The greater capillary density as a consequence of endurance training, would also be expected to decrease the time taken for lactate to diffuse across the interstitial space. We expect that these variables would have had little effect on the distribution of lactate, since measurements were made over several minutes in this investigation.

One of the early adaptations to almost any level of aerobic exercise is an approximate 30% increase in plasma volume (Rocher, Kirsch, & Stroboy, 1976), and a rise in plasma volume could increase the lactate distribution space. By not taking a rise in lactate distribution volume into account, it may be that the lactate Ra values after training are over-estimated and that the training induced decreases in lactate appearance may actually be greater than measured.

Conversely, the decreases in lactate Ra and Rd after training could be less than estimated. A large part of the reduction in lactate turnover may be due to the lower rates of carbohydrate oxidation at sub-maximal VO_2's after training (Figure 4.4). Less pyruvate delivery to the mitochondria would decrease the net transfer of isotope from ^{14}C-lactate to pyruvate and reduce the apparent lactate Ra and Rd values. Unfortunately, the extent to which isotope equilibration between ^{14}C-lactate and pyruvate, via the lactate permease and lactate dehydrogenase equilibria, influences the estimates of lactate turnover and oxidation is difficult to assess. In this study, some pyruvate would also have been labeled by the progressive transfer of ^{14}C from lactate to glucose. The only indication of how glucose turnover might have affected the estimates of lactate turnover, as a consequence of training, is provided by Coggan et al. (1990). They showed that training decreased the turnover of glucose, at a VO_2 of 1.9 $l.min^{-1}$, from 100 to 72 umol C_3 $units.min^{-1}.kg^{-1}$. In this study,

the equivalent lactate Ra and Rd values were reduced from around 140 and 110 to 80 umol.min^{-1}.kg^{-1}. Thus, the lower lactate Ra and Rd values after training have to be considered in relation to the decrease in overall carbohydrate oxidation.

One explanation for the lower blood lactate Ra values during submaximal exercise after endurance training is that muscle lactate production was decreased. Several studies have shown that endurance training reduces carbohydrate utilization (Hermansen, 1971; Hickson, et al., 1977; Rennie, et al., 1976; Saltin, et al., 1976) and muscle lactate accumulation (Henriksson, 1977; Karlsson, Nordesjo, Jorfeldt, & Saltin, 1972; Saltin, et al., 1976) during exercise.

Evidence for a decrease in carbohydrate utilization during sub-maximal exercise in the trained state is provided by the more gradual rise in respiratory exchange ratio with increasing VO_2 (Hermansen, 1971; Hickson, et al., 1977; Rennie, et al., 1976; Saltin, et al., 1976). In our subjects, we also found that respiratory exchange ratios (RER) were lower during exercise after training (Figure 4.4).

Underlying the slower rates of glucose and glycogen metabolism is probably the increased mitochondrial content of the trained muscle (Holloszy, et al., 1984). As demonstrated by Dudley et al. (1987), an elevated mitochondrial density improves respiratory control sensitivity. With a lower cytosolic ADP concentration required for any given rate of oxidative phosphorylation, the activation of carbohydrate utilization by the displacement of the creatine kinase and adenylate kinase equilibria is decreased and the contribution to ATP production from free fatty acid oxidation is proportionately increased.

A reduced breakdown of glucose and glycogen during exercise might also account for the accelerated disappearance of the higher (> 4 mmol.l^{-1}) blood lactate concentrations after training (Figure 4.3 B). During human exercise, approximately 75% of the carbon from the lactate turning over is converted to pyruvate and NADH by the cytoplasmic lactate dehydrogenase equilibrium and oxidized in the mitochondria (Mazzeo, et al., 1986). Presumably, in that investigation, some of the carbon was from lactate and some from glucose labeled by lactate. With less competition from glycolytic pyruvate and NADH formation, more of the pyruvate and NADH generated by the lactate dehydrogenase reaction would be expected to be oxidized.

Changes in catecholamine accumulation are another explanation for a decreased lactate production. Epinephrine is a major effector of lactate production, through its modulation of muscle glycogenolysis and glycolysis (Stainsby, Sumners, & Eitzman, 1985). Three weeks of endurance training results in decreases in plasma catecholamine (Winder, et al., 1979) as well as blood lactate concentrations (Gaesser, et al., 1986) during submaximal exercise. A recent study showed high correlations between lactate Ra and arterial epinephrine levels during both rest and exercise (Brooks, et al., 1991). Therefore, the lower blood lactate concentrations observed in trained compared to untrained individuals during submaximal exercise, are likely partly due to a decreased effect of epinephrine on muscle glycogenolysis.

Curvilinear rises in Rd values with increasing blood lactate concentrations have also been reported by Stanley et al. (1985). In their 6 subjects, the disappearance of approximately 2.0 mmol. l^{-1} arterial lactate concentrations occurred at a rate of around 52 - 55 umol.min^{-1}.kg^{-1}. At similar (1.8 mmol. l^{-1}) venous lactate concentrations, we found Rd values to be in the region of 71 umol. min^{-1}. kg^{-1}. The differences between our blood lactate Rd values

and those of Stanley et al. (1985) may be due to differences in exercise protocol. Whereas our subjects exercised in the upright position, their subjects performed supine cycle ergometry. Folkow et al. (1971) noted that the calf blood flow in man could be 50 to 60% larger when heavy exercise was performed in the upright position as compared to the reclining position, thereby improving the possibilities for lactate removal. These differences in lactate Rd could also be explained by the fact that we used venous, rather than arterial blood sampling. When blood is sampled from a vein, instead of from an artery, there is up to a 30% decrease in lactate specific radioactivity, produced by the larger volume of tissue perfused (Freminet, & Minaire, 1984; Reilly, & Chandrasena, 1977). A greater dilution of labeled lactate by unlabeled lactate would increase calculated Ra and Rd.

Lactate Rd was reduced at all corresponding VO_2 measurements during submaximal exercise after training (Figure 4.2 B). We attribute this finding to the lower lactate Ra's and blood [La]'s during exercise after training. Lactate Rd is a function of lactate Ra and arterial [La], which is indirectly related to metabolic rate (Stanley, et al., 1985).

In summary, the diminished lactate concentration seen during exercise at equivalent and relative metabolic rates, was the consequence of a combined process after training. During the initial stages of graded exercise when work rates were low, decreased lactate production likely caused a reduced lactate Ra as a result of mass action (Connett, et al., 1990). During exercise at these lower absolute work rates, lactate clearance was not improved by training, but was higher at equivalent relative work rates. During the later stages of graded exercise, when work rates were high and lactate Ra's became similar, lactate concentration remained low due to a sustained higher rate of lactate clearance after training.

CHAPTER 5

EFFECTS OF TRAINING ON LACTATE REMOVAL BY OXIDATION AND GLUCONEOGENESIS DURING EXERCISE

INTRODUCTION

Recent studies have demonstrated that most of the lactate produced during sustained submaximal exercise in rats (Donovan & Brooks, 1983), dogs (Depocas, Minaire, & Chatonnet, 1969; Issekutz, 1984; Issekutz, Shaw, & Issekutz, 1976), and humans (Mazzeo, et al., 1986; Stanley, et al., 1985; Stanley, et al., 1986; Stanley, et al., 1988) is oxidized. Brooks has proposed a lactate shuttle hypothesis, in which lactate formed in active muscle fibres exhibiting high rates of glycogenolysis and glycolysis, is utilized as an energy source in adjacent fibres with higher respiratory capacities (Brooks, 1985; Brooks, 1986b). In addition, the lactate formed in highly active muscle is also transported via the blood to the heart (Carlsten, et al., 1961; Gertz, et al., 1981) and less active skeletal muscle beds for oxidation (Jorfeldt, 1970; Stainsby, & Welch, 1966; Stanley, et al., 1986).

Some blood lactate also acts as a gluconeogenic precursor in the liver (Cori, 1931) and renal cortex (Krebs, 1964; Levy, 1962). Recent studies by Foster (1984), Katz and McGarry (1984), and Newgard et al. (1983) have shown that lactate is particularly important in the post-prandial synthesis of hepatic glycogen via gluconeogenesis in animals. During moderate exercise in humans, it has been estimated that around 25% of the lactate produced is converted to glucose (Mazzeo, et al., 1986; Stanley, et al., 1985; Stanley, et al., 1986; Stanley, et al., 1988).

In the previous chapter it was shown that the lower blood lactate concentrations during progressive exercise after training are a combined effect of a decreased rate of lactate appearance (Ra) at low work rates and an increased rate of lactate clearance (MCR) at high work rates . Here it is shown that the improved lactate rate of disappearance after training is due to an increased lactate removal by oxidation rather than gluconeogenesis.

RESULTS

^{14}C- lactate removal via oxidation

No effect of training was observed on the oxidative clearance of ^{14}C-lactate (l. min^{-1}) at equivalent metabolic rates (Figure 5.1 A). Whereas training had no effect on the oxidative clearance of lactate at equivalent metabolic rates, it did increase the direct and indirect oxidation of equivalent lactate concentrations (Figure 5.1 B). It should be emphasized however that these data be interpreted with caution. For instance, the similar ^{14}C oxidative clearance vs. VO_2 curves in Figure 5.1 A may in part result from the lower lactate concentrations at equivalent VO_2's after training (Table 5.1). Likewise, some of the improvement in ^{14}C oxidative clearance in Figure 5.1B could be due to the higher metabolic rates at any given lactate concentration after training (Table 5.1).

The estimated rates of ^{14}C-lactate oxidation (lac R_{ox}), corrected for the predicted $V^{14}CO_2$ from ^{14}C-glucose oxidation as described in the Methods, are shown in Figures 5.2 A and 5.2 B. Lac R_{ox} was decreased at VO_2's of 1.06 to 2.27 $l.min^{-1}$ after training (Figure 5.2 A, $p < 0.01$), but was significantly higher at equivalent venous lactate concentrations [La]'s after training (Figure 5.2 B).

TABLE 5.1. EFFECTS OF TRAINING ON SELECTED CHARACTERSITICS OF THE SUBJECTS

		Before-training	After-training
VO_2max (l. min^{-1})		2.59 ± 0.1	3.45 ± 0.2 *
[Lactate] (mmol. l^{-1})	rest	1.19 ± 0.1	1.00 ± 0.1
	peak	7.44 ± 0.8	6.03 ± 1.1
Lactate Ra	rest	18.6 ± 1	18.1 ± 2
(umol. $min^{-1}.kg^{-1}$)	peak	214.5 ± 28	243.8 ± 12
Lactate Rd	rest	18.3 ± 1	18.4 ± 2
(umol. $min^{-1}.kg^{-1}$)	peak	177.1 ± 25	224.5 ± 11 *
Lactate MCR	rest	15.6 ± 1	18.4 ± 1 *
(ml. min^{-1}. kg^{-1})	peak	31.3 ± 4	40.2 ± 3 †

Values are means ± S.E. determined during the 40W. 6 min^{-1} progressive exercise test for 8 subjects. The VO_2max values were determined on a 20W. min^{-1} progressive exercise test (* and † denotes differences significant at $p < 0.05$ and $p < 0.01$ respectively).

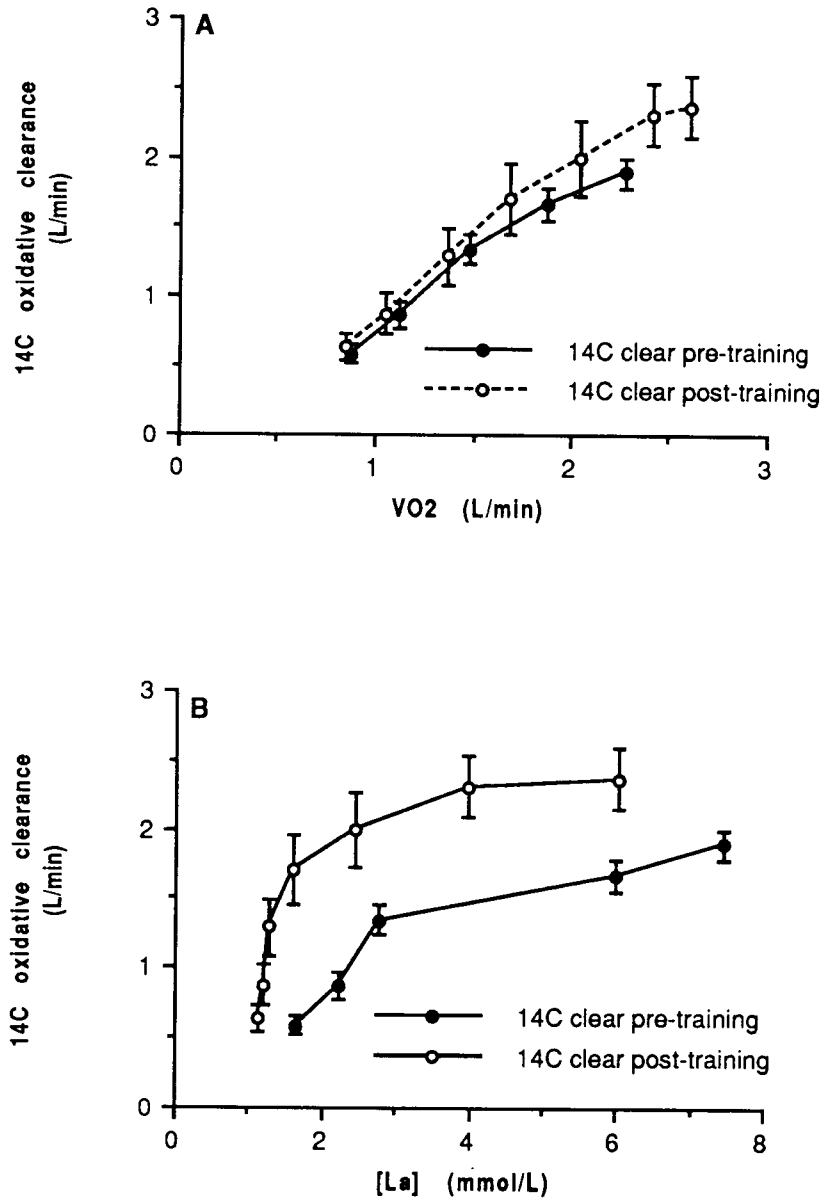


FIGURE 5.1. Effect of training on the oxidative clearance of ^{14}C at equivalent metabolic rates (panel A; no training effect observed), and on the direct and indirect oxidation of ^{14}C at equivalent lactate concentrations (panel B; oxidative clearance was increased at blood [La]'s of 1.6 mmol.l^{-1} and higher, $p < 0.01$). Values shown are the mean ± S.E. of 8 subjects.

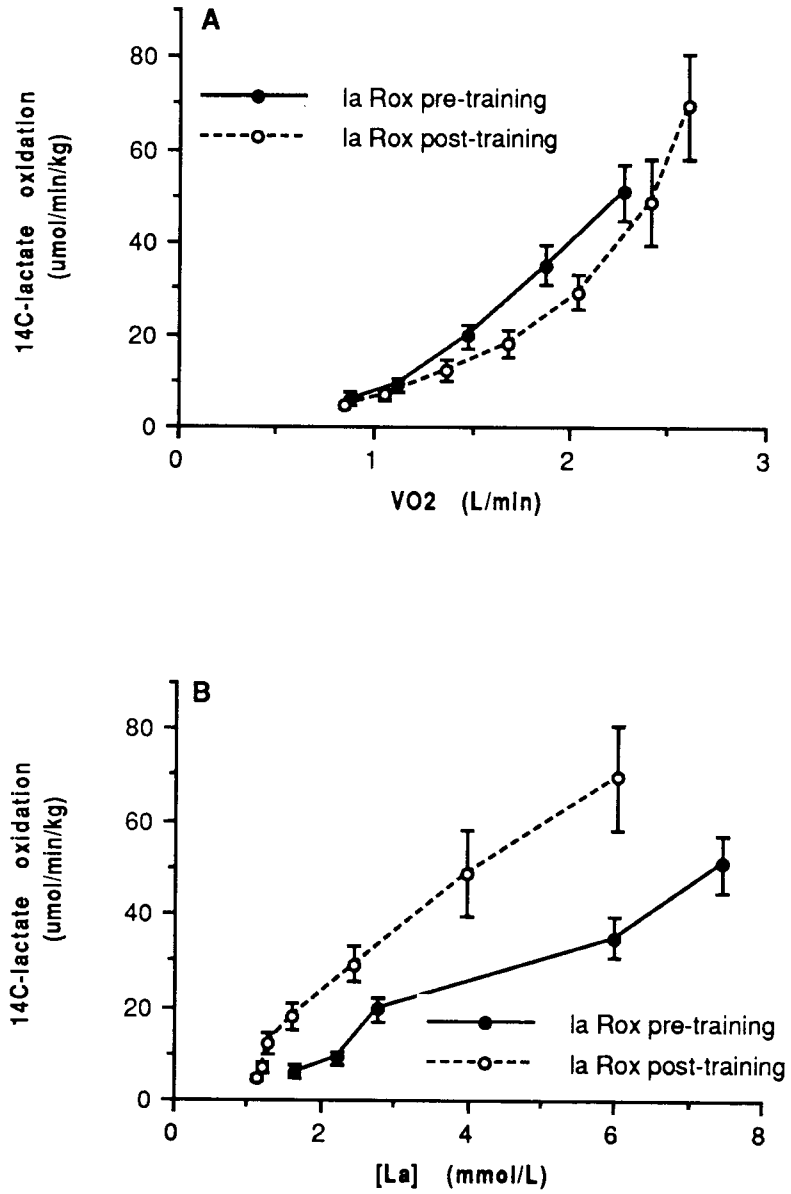


FIGURE 5.2. Effect of endurance training on ^{14}C-lactate oxidation at equivalent metabolic rates (panel A; lac R_{ox} was reduced at VO_2's of 1.37 to 2.27 l.min^{-1} after training, $p < 0.01$), and blood lactate concentrations (panel B; lac R_{ox} increased at blood [La]'s of 1.6 mmol.l^{-1} and higher after training, $p < 0.01$).

^{14}C-lactate removal by gluconeogenesis

In contrast to the ^{14}C-lactate removal by oxidation findings, the transfer of ^{14}C-label from equivalent concentrations of lactate to glucose via gluconeogenesis was unaffected by training. Curves showing the percentage of glucose labeled by ^{14}C-lactate with increasing VO_2 and lactate concentration are presented in Figures 5.3 A and 5.3 B. The lower percentages of glucose labeled with increasing VO_2 in Figure 5.3 A, are presumed to be due to the lower blood lactate concentrations after training. Gluconeogenesis is regulated by both substrate availability and metabolic rate (Ahlborg, & Felig, 1982; Ahlborg, Felig, Hagenfeldt, Hendler, & Wahren, 1974). No training effect on the percentage of glucose labeled was observed with increasing lactate concentrations (Figure 5.3 B).

The transfer of ^{14}C-label from lactate to glucose only provides an approximate indication of the rate of gluconeogenesis. With progressive exercise, isotope dilution by carbon turnover in the tricarboxylate cycle may decrease as the conversion of lactate to glucose is accelerated (Donovan & Brooks, 1983). Because isotope dilution in the tricarboxylate cycle during exercise is not known, the removal of ^{14}C-lactate via gluconeogenesis (lac R_{glu}) in umol.min^{-1}.kg^{-1} was estimated from the difference between the umol.min^{-1}.kg^{-1} lac R_{ox} values in Figure 5.2 and the overall umol.min^{-1}.kg^{-1} rates of lactate disappearance (lac R_d) in Table 5.1. Changes in lac R_{glu} with increasing VO_2 and blood lactate concentration are shown in Figures 5.4 A and 5.4 B. Lac R_{glu}, when expressed relative to venous [La], was not different before and after training (Figure 5.4 B). Lac R_{glu} was however significantly lower at equivalent metabolic rates after training at VO_2's of 1.06 to 2.27 l. min^{-1} (Figure 5.4 A, $p < 0.01$).

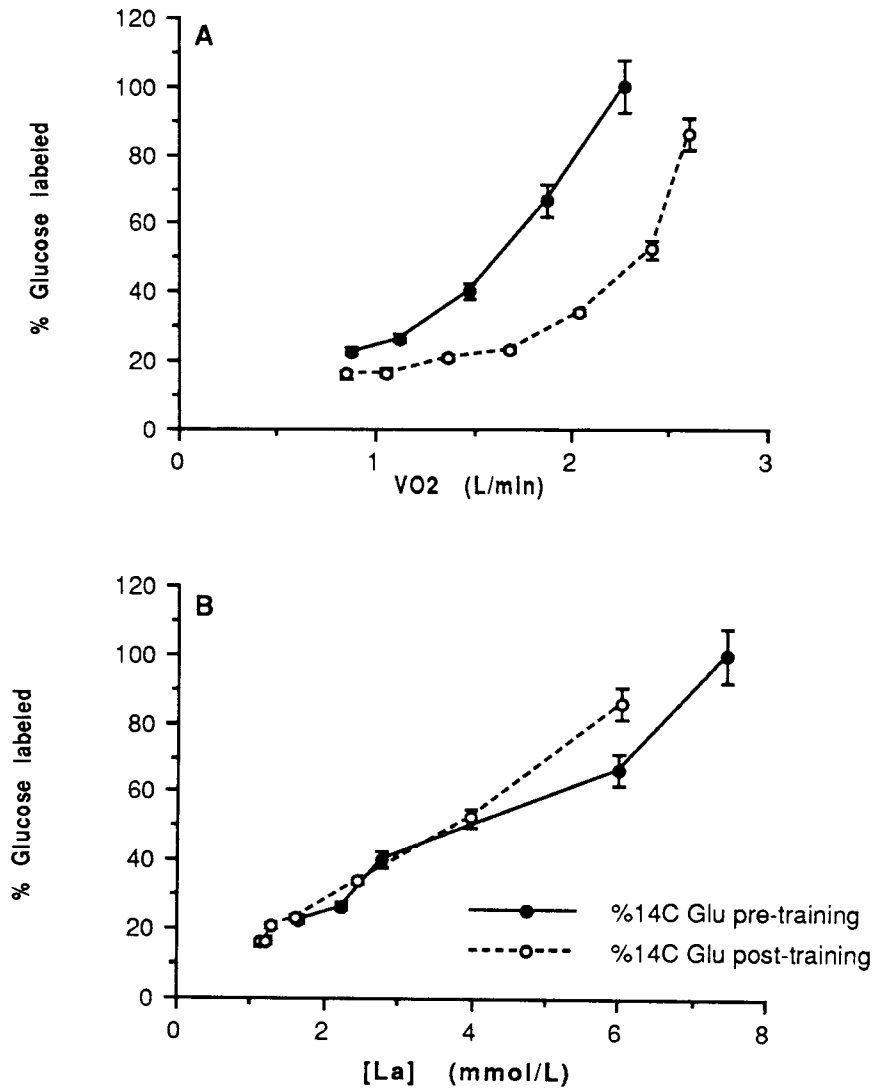


FIGURE 5.3. Effects of endurance training on the percentage of glucose labeled by ^{14}C-lactate with increasing metabolic rate (panel A; % glucose labeled was lower after training at VO_2's of 0.85 l.min^{-1}, $p < 0.05$, and VO_2's of 1.06 l.min^{-1} and higher, $p < 0.01$), and blood lactate concentration (panel B; no effect of training observed).

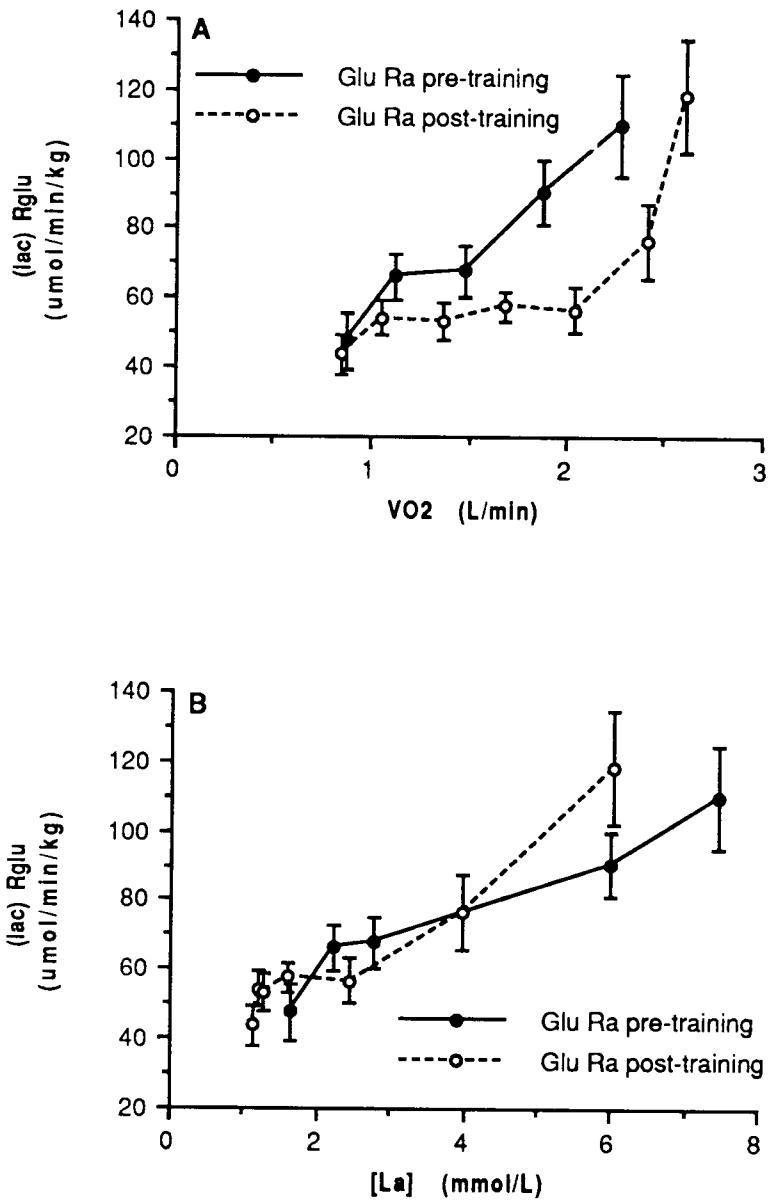


FIGURE 5.4. Effects of endurance training on the estimated removal of ^{14}C-lactate via gluconeogenesis with increasing metabolic rate (panel A; lac Rglu was lower after training at VO_2's 1.37 to 2.27 l.min^{-1}, $p < 0.01$), and blood lactate concentration (panel B; no effect of training observed).

TABLE 5.2. BLOOD METABOLITE AND HORMONE CONCENTRATIONS, AND CHANGES IN MUSCLE GLYCOGEN CONTENTS DURING EXERCISE BEFORE AND AFTER TRAINING

		Before-training	After-training
Glucose ($mmol.l^{-1}$)	rest	5.5 ±0.3	5.7 ± 0.2
	peak	4.9 ± 0.7	5.9 ± 0.3
Glucagon ($pg.ml^{-1}$)	rest	28.8 ± 4.3	26.9 ± 5.9
	peak	31.6 ± 7.8	43.1 ± 7.2 *
Insulin ($iU.ml^{-1}$)	rest	7.3 ±1.4	6.5 ± 1.0
	peak	5.2 ± 1.2	6.4 ± 0.9
FFA ($mmol.l^{-1}$)	rest	0.32 ± 0.02	0.17 ± 0.01 **
	peak	0.25 ± 0.01	0.12 ± 0.03 *
Muscle glycogen (mM/kg wet wt.)	rest	111.1 ± 10.2	157.8 ± 12.3
	post-exercise	73.8 ± 11.3	130.8 ± 8.0
Glycogen depletion (mM/kg wet wt.)		37.2 ± 6.3	27.0 ± 6.9

Values are means of 8 subjects ± S.E.; * $p < 0.05$ and ** $p < 0.01$ are differences between resting and peak values. All muscle glycogen contents at rest and post-exercise before and after training were different ($p < 0.05$), but total depletion of glycogen was not altered by training.

Overall carbohydrate oxidation

The percentage of carbohydrate utilized during exercise after training, was reduced at all VO_2's (Figure 5.5 A, $p< 0.01$). The blood metabolite and hormone concentrations and the changes in muscle glycogen in Table 5.2 are also consistent with decreased carbohydrate oxidation after training. Plasma FFA concentrations were lower at rest ($p< 0.01$) and at fatigue after-training (Table 5.2, $p< 0.05$). Plasma glucagon increased as expected from resting values during exercise, this increase only being significant after training. Peak glucagon concentrations were also higher after training (Table 5.2, $p< 0.05$). Plasma glucose and insulin concentrations did not change from rest to peak exercise values either before or after training, and were not different when compared before or after-training (Table 5.2). Muscle glycogen concentrations were significantly higher after training, both before and after exercise (Table 5.2, $p < 0.05$). Muscle glycogen depletion was 37 .2 before and 27 $mmol.kg^{-1}$ wet weight after training. This accounted for a 27% sparing of muscle glycogen after training ($p< 0.05$).

Overall CHO oxidation was reduced at VO_2's of 1.06 to 2.27 $l.min^{-1}$ after training (Figure 5.5 B, $p < 0.01$). By 30 min of exercise, CHO oxidation decreased from 190 ± 10 umol $glucose.min^{-1}.kg^{-1}$ before training, to 145 ± 7 umol $glucose.min^{-1}.kg^{-1}$ after training, or from 3.0 ± 0.2 to 2.2 ± 0.1 grams ($p < 0.01$). The finding of reduced rates of lac R_{ox} and lac R_{glu} (Figures 5.2 A and 5.4 A respectively) support this finding.

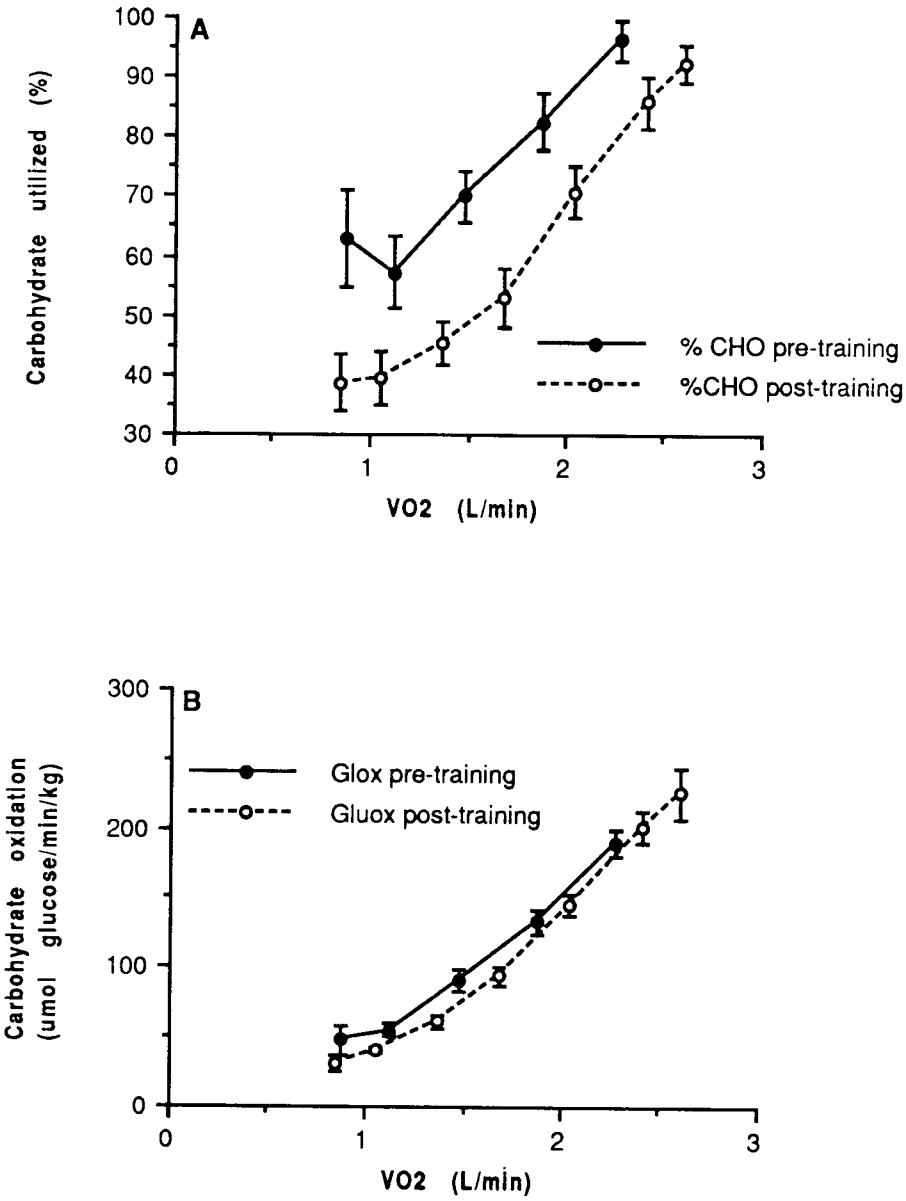


FIGURE 5.5. Panel A shows the effect of endurance training on the percentage carbohydrate utilization with increasing metabolic rate; carbohydrate utilization was lower at VO_2's of 0.85 to 2.27 $l.min^{-1}$ after training ($p < 0.01$). Panel B illustrates overall carbohydrate oxidation with increasing metabolic rate; oxidation was lower at VO_2's of 1.06 to 2.27 $l.min^{-1}$ after training ($p < 0.01$).

Discussion of Training Effects on Lactate Removal by Oxidation and Gluconeogenesis

This study revealed two major findings related to the removal of lactate during exercise after endurance training in humans. One is that the removal of lactate via oxidation, rather than gluconeogenesis, is improved by endurance training. Another is that the estimated rates of gluconeogenesis from lactate are much higher than those previously reported.

Several authors have postulated (Donovan & Brooks, 1983; Stanley, et al., 1984; Stanley, et al., 1988) that at low to moderate exercise intensities, the major fate of the lactate produced during the activity is oxidation. In addition, every study that has determined lactate disposal during exercise has reported a direct relationship between lactate disposal rate and metabolic rate as given by the oxygen consumption (Stainsby, & Brooks, 1990). In this investigation we also demonstrated that the oxidative clearance of ^{14}C-lactate (Figure 5.1 A) and the rate of lactate oxidation (Figure 5.2 A) increased with increases in oxygen consumption. The finding that the oxidative clearance of ^{14}C-lactate at equivalent metabolic rates was not affected by training (Figure 5.1 A) needs to be interpreted with caution, as indicated in the methods. This estimate of lactate oxidation may not be sensitive enough to detect changes in oxidation of lactate, particularly since the estimated rate of lactate oxidation was significantly decreased both at low (VO_2's of 1.06 l.min^{-1}), and higher metabolic rates (VO_2's of 2.27 l.min^{-1}) after training, from 9.0 ± 1.4 to 7.1 ± 1.3 umol.min^{-1}.kg^{-1}, and from 50.9 ± 6.1 to 29.3 ± 3.7 umol.min^{-1}. kg^{-1} respectively (Figure 5.2 A). The observation that training does not improve the oxidation of lactate at equivalent metabolic rates has also been observed in rats. Donovan and Brooks (1983) demonstrated that lactate oxidation was not significantly different during both easy and heavy exercise in trained and untrained animals.

When expressed at equivalent venous lactate concentrations however, lac R_{ox} was significantly higher at lactate concentrations of around 1.5 mmol. l^{-1} and higher after training (Figure 5.1 B and 5.2 B). It has been shown in humans that the exercising skeletal muscle is a site which contributes to the oxidative removal of lactate (Jorfeldt, 1970). It was also recently observed that the exercising legs are responsible for about half of the lactate cleared from the blood during moderate-intensity exercise (Stanley, et al., 1986). One of the metabolic consequences of endurance training is an increase in the skeletal muscle mitochondrial density and oxidative capacity (Holloszy, et al., 1984). The improved rates of lactate oxidation at equivalent venous lactate lactate concentrations after training, could be due to the increase in oxidative capacity of skeletal muscle resulting from endurance training.

The importance of lactate as an oxidizable substrate in overall carbohydrate utilization can be determined from the total rates of carbohydrate oxidation (Figure 5.5 B), the lactate $R_{ox}/2$ (Figure 5.2 A), and the lactate $R_{glu}/2$ (Figure 5.4 A). At test termination after training (approximately 75% VO_2max), these values revealed that 42% of the carbohydrate utilized in the final 3 min of exercise, was coming from the direct and indirect oxidation of lactate. At this stage, the total rate of carbohydrate oxidation was around 3.5 $g.min^{-1}$ or 226 umol $glucose.kg^{-1}$, and the estimated lac $R_{ox}/2$ and lac $R_{glu}/2$ were 35 and 59 umols $glucose.min^{-1}.kg^{-1}$ respectively. The approximate 15% contribution of lac R_{ox} to the overall carbohydrate oxidation is in good agreement with the estimated 11% contribution in a group of fit men studied during cycling by Mazzeo et al. (1986).

The lower rates of lactate oxidation at equivalent metabolic rates after training (Figure 5.2 A), can be explained by the lower venous lactate concentrations observed after training at equivalent metabolic rates (Table 5.1). In addition, we found that the overall rate of

carbohydrate oxidation was decreased at equivalent metabolic rates after training (Figure 5.5 B), with supporting evidence for reduced carbohydrate oxidation being derived from decreased muscle glycogen usage, lower FFA levels at exhaustion, and higher peak glucagon concentrations (Table 5.2).

Although the percentage of lactate oxidized at rest has been well-documented in humans, we are only aware of one study in which lactate oxidation was estimated during exercise, and none in which the effect of training on lactate oxidation has been studied. Mazzeo et al. (1986) determined the disposal of blood lactate via oxidation during cycling endurance exercise demanding 50% VO_2max and 75% VO_2max. We estimated lactate removal via oxidation in our untrained subjects at 75% VO_2max to be 3.6 $mg.min^{-1}.kg^{-1}$, and after training to be 5.9 $mg.min^{-1}.kg^{-1}$. These estimates are in good agreement with those of Mazzeo et al. They found that the rate of lactate removal via oxidation in their fit subjects was approximately 4.2 $mg.min^{-1}.kg^{-1}$ at 75% VO_2max. One possible explanation for the higher lactate R_{OX} in our subjects after training is the greater blood lactate concentrations arising from progressive rather than steady-state exercise. Some increase in estimated lac R_{OX} values might also arise from our use of venous, rather than arterial, blood sampling. As mentioned previously, with sampling of venous blood, there is up to a 30% decrease in lactate specific radioactivity produced by the larger volume of tissue perfused (Freminet, et al., 1984; Reilly, et al., 1977). A lower lactate specific radioactivity would increase calculated lactate R_{OX} values.

It should, however, be emphasized that the data be regarded as more directional than absolute. The assumption of a 2 : 1 lactate to glucose oxidation ratio is based on data obtained during low (43% of VO_2max) intensity exercise, where blood lactate concentrations remained at around 1 $mmol.l^{-1}$ (Stanley, et al., 1988). In our progressive

exercise tests, the rising concentrations of lactate would be expected to have increased the lactate to glucose oxidation ratio.

To illustrate how a higher lactate to glucose oxidation ratio might affect the findings, we compared the corrected and uncorrected lac R_{ox} values. At a VO_2 of 1.06 l.min^{-1} before training the corrected vs. uncorrected lac R_{ox} was 9.0 ± 1.4 vs. 10.3 ± 1.6 umol.min^{-1}.kg^{-1}. At a VO_2 of 2.27 l.min^{-1} before training the corrected vs. uncorrected lac R_{ox} was 50.9 ± 6.1 vs. 73.5 ± 10.5 umol.min^{-1}.kg^{-1}. Similar differences were also observed in corrected vs. uncorrected lac R_{ox} values after training. It can be concluded from these observations, that at the higher metabolic rates (~ 75% VO_2max), use of the uncorrected lac R_{ox} values would overestimate rates of lac R_{ox} , and the rates of lac R_{glu} would be accordingly underestimated.

The effects of endurance training on gluconeogenesis in man until now have not been determined. We found that the percentage of glucose labeled by lactate at equivalent metabolic rates was significantly decreased by training (Figure 5.3 A). Coggan et al. (1990) showed that endurance training in men decreased the turnover and oxidation of plasma glucose during prolonged exercise at 60% VO_2max, suggesting at least in part, less activation of hepatic glycogenolysis and/or gluconeogenesis during exercise after training. In our subjects we estimated that at 60% VO_2max after training, lac R_{glu} was 5.4 mg.min^{-1}.kg^{-1}, or 0.5 g.min^{-1}. These after training R_{glu} values are in accord with the 0.5 g.min^{-1} glucose rate of appearance reported in Coggan et al's trained subjects. Prior to training, the estimated lac R_{glu} at 60% VO_2max in our subjects was around 8.1 mg. min^{-1}.kg^{-1}, or 0.7 g.min^{-1}. This latter value, as well as the lac R_{glu} values before and after training at exhaustion (110 and 118 umol.min^{-1}.kg^{-1} respectively), are much higher

than previously reported values. However, these high lac R_{glu} values were only seen when blood lactate concentrations began to rise beyond 4-5 mmol.l^{-1}.

The effect of endurance training on liver enzymes has been examined by several investigators (Galbo, Saugmann, & Richter, 1979; Huston, Weiser, Dohm, Askew, & Boyd, 1975; Zawalich, Maturao, & Felig, 1982). The universal finding of these studies was that endurance training had no major effect on the liver content of gluconeogenic or glycogenolytic enzymes. It is a reasonable assumption therefore, that although the liver is quite active during exercise, no intrinsic metabolic change in liver activity can explain the reduction in percentage of glucose labeled by ^{14}C, or the reduction in lac R_{glu} at equivalent metabolic rates in our subjects after training.

Glucose production is regulated by both the availability of gluconeogenic precursors (Ahlborg, et al., 1982; Ahlborg, et al., 1974) and the metabolic rate (Dohm, Kasperek, & Barakat, 1985). Although we estimated that gluconeogenesis increased with increasing metabolic rates, both the percentage of glucose labeled by ^{14}C-lactate (Figure 5.3 A), and the lac R_{glu} (Figure 5.4 A), were reduced at equivalent metabolic rates after training. The delivery and uptake of gluconeogenic precursors are, perhaps, a more important stimulus for gluconeogenesis than the metabolic rate. We found that venous blood lactate concentrations were significantly lower at equivalent metabolic rates after training (Table 5.1). The lower venous blood lactate concentration after training could potentially reduce the stimulation of gluconeogenesis, resulting in lower estimated gluconeogenic rates in our subjects. Rates of lactate uptake have been shown to be proportional to arterial concentration in the heart (Olson, & Piatnek, 1959), kidneys (Levy, 1962), and hepatic-splanchnic tissues (Rowell, et al., 1966). The observation of an increase in estimated gluconeogenesis both before and after training with rises in venous lactate concentration

(Figure 5.3 B and 5.4 B) supports this contention, although no difference was observed in the estimated rates of gluconeogenesis as a result of endurance training.

In contrast to these findings, Donovan and Brooks (1983) using rats, demonstrated increased gluconeogenic rates in trained animals, possibly due to less diversion of blood away from gluconeogenic organs at high work rates . More recently, Donovan and Sumida (1990b) also using rats, demonstrated an elevated glucose production in trained animals, suggesting a training-enhanced gluconeogenic capacity. The results of the animal studies conflict with our findings in humans, suggesting that the effects of training on the production and removal of lactate may be species-dependent.

An important additional finding in this study was that the estimated rates of gluconeogenesis from lactate are much higher than those previously reported. Stanley et al. (1988), during moderate intensity (43% VO_2max; VO_2 = ~2.0 $l.min^{-1}$) supine cycle ergometry in trained men, showed lactate to glucose conversion rates of approximately 0.83 $mg.min^{-1}.kg^{-1}$, where the estimated rate of gluconeogenesis represented approximately 24% of the lactate rate of disappearance. At similar metabolic rates (corresponding to 77% and 59% of the before and after training VO_2max in our subjects), we estimated that 70% and 62% of the lactate rate of disappearance respectively, could be attributed to lac R_{glu}. The respective before and after training lac R_{glu} values were 8.5 $mg.min^{-1}.kg^{-1}$ and 4.5 $mg.min^{-1}.kg^{-1}$, the latter value being in good agreement, as demonstrated previously, with the glucose rate of appearance reported by Coggan et al. (1990). The most reasonable explanation for the difference between our values and those of Stanley et al., could be due to the different blood lactate concentrations at this particular metabolic rate. Stanley et al.'s subjects maintained blood lactate concentrations of around 1 $mmol.l^{-1}$. In our subjects, before and after training

venous lactate concentrations at a VO_2 of around 2 $l.min^{-1}$ were approximately 6 $mmol.l^{-1}$ and 2 $mmol.l^{-1}$ respectively.

In summary, at equivalent metabolic rates after training, lactate removal via gluconeogenesis and oxidation is reduced. At equivalent blood lactate concentrations after training, only the removal of lactate via oxidation is significantly improved.

CHAPTER 6

EFFECTS OF TRAINING ON RESPIRATORY RESPONSES DURING PROGRESSIVE EXERCISE

INTRODUCTION

Continuous changes in blood lactate production and degradation have recently been demonstrated during incremental exercise (Stanley, et al., 1985). These findings, using radioisotopes, are contrary to those which suggest a sudden change in blood lactate concentration [La^-] with increasing intensity of exercise (Beaver, Wasserman, & Whipp, 1985; Gladden, Yates, Stemel, & Stamford, 1985; Jones, & Ehrsam, 1982), the so called lactate threshold (Beaver, et al., 1985; Davis, et al., 1983; Gladden, et al., 1985) or anaerobic threshold (Wasserman, et al., 1973). The anaerobic threshold describes the oxygen uptake (VO_2) at which ventilation (V_I) appears to deviate from linearity, with lactic acidosis being implicated in the observed phenomenon (Hughes, et al., 1968; Hughson, & Green, 1982; Jones, 1984; Sutton, & Jones, 1979; Whipp, & Ward, 1980).

Recent studies have shown that both muscle (Connett, et al., 1990; Stainsby, et al., 1989) and blood (Green, Hughson, Orr, & Ranney, 1983; Hogan, Cox, & Welch, 1983; Hughson, et al., 1987; Yeh, Gardner, Adams, Yanowitz, & Crapo, 1983) lactate concentrations increase as continuous exponential functions of oxygen consumption. A study by Dennis et al. (Dennis, et al., 1991) showed that V_I increases exponentially with increasing work rates. This finding is contrary to the concept of a non-linear, or threshold increase in V_I with increasing VO_2. Furthermore, changes in blood [La^-] can be dissociated

from changes in V_I during incremental exercise (Hagberg, et al., 1982; Heigenhauser, Sutton, & Jones, 1983; Hughes, Turner, & Brooks, 1982).

Campbell et al. (1989) recently investigated the use of different ramp exercise slopes on the changes in blood [La^-] during exercise. Their investigation supported the findings of an exponential increase in blood [La^-] during incremental exercise. However, the effects of training on blood [La^-] and V_I responses during incremental exercise of different ramp slopes has not been investigated. The purpose of this investigation was therefore to determine if training had any effect on the inter-relationships between the rises in V_I, the increases in CO_2 production (VCO_2) and blood [La^-] during incremental exercise. These relationships were investigated using different ramp exercise slopes, to determine if the exercise protocol contributed to alterations in the metabolic response.

RESULTS

Effects of exercise ramp on heart rate and gas exchange

Heart rate and VO_2 vs. work rate

Fast ramp protocol. Heart rate (Figure 6.1 A) and VO_2 (Figure 6.1 C) increased linearly with increases in work rate. The differences in linear vs. exponential equation R^2 HR and VO_2 vs work rate values are shown in table 6.1. Heart rate was significantly lower at rest, and at all workloads higher than 60W after training ($p < 0.05$). Rises in VO_2 were not different before or after training.

Slow ramp protocol. Heart rate (Figure 6.1 B) and VO_2 (Figure 6.1 D) increased exponentially with increasing work rate before and after training. The differences in linear vs. exponential equation R^2 HR and VO_2 vs work rate values are shown in table 6.1.

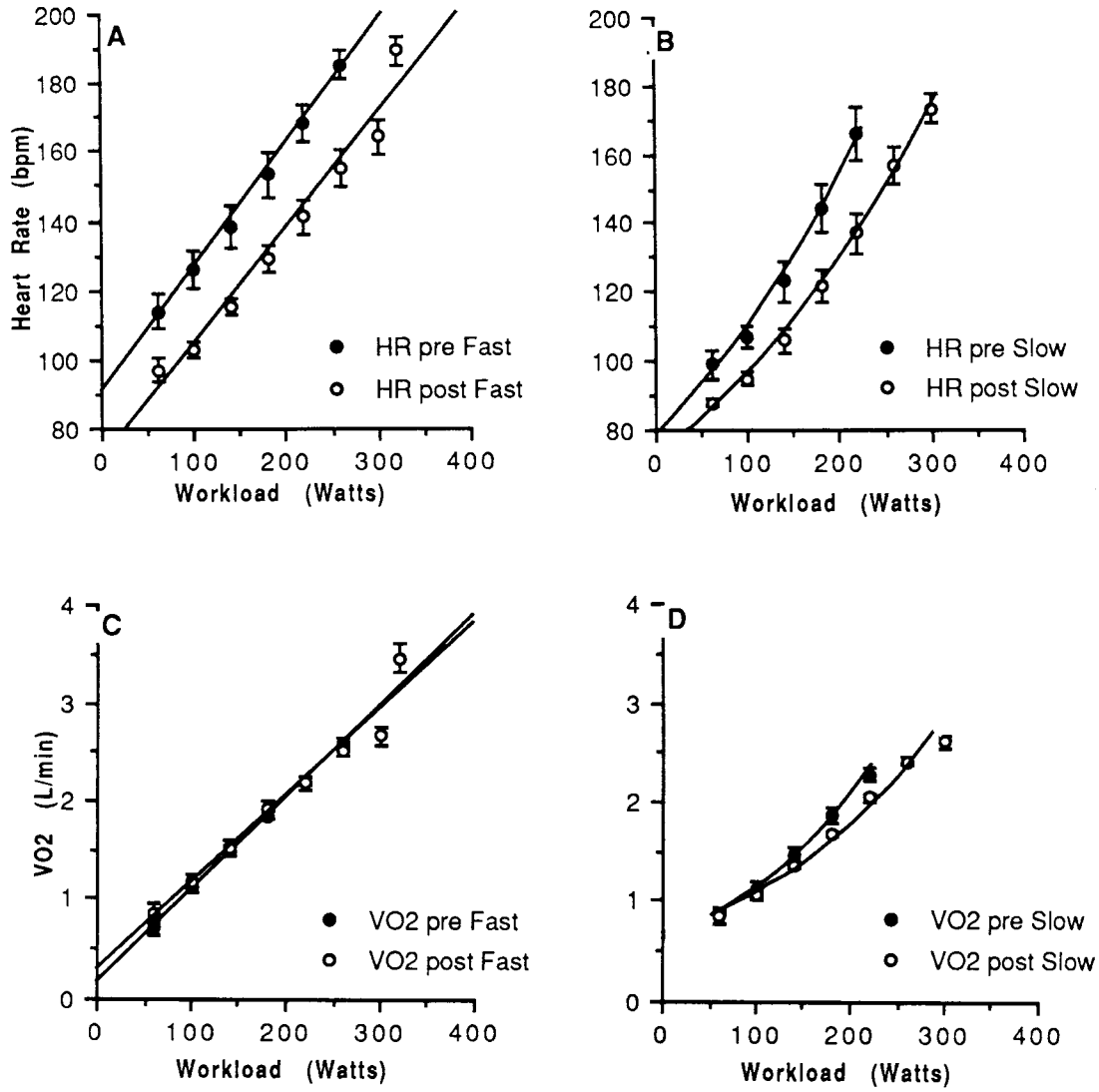


FIGURE 6.1. The effect of endurance training and exercise ramp on the rises in heart rate (HR) and VO_2 with increasing workload. HR (panel A; $R^2 = 0.981$ before and 0.977 after) and VO_2 (panel C; $R^2 = 0.975$ before and 0.971 after), increased linearly in the fast ramp. In contrast HR (panel B; $R^2 = 0.981$ before and 0.994 after) and VO_2 (panel D; $R^2 = 0.989$ before and 0.994 after), were best described by exponential rate equations in the slow ramp before and after training ($p < 0.05$). Values shown are means ± S.E. for the 8 subjects.

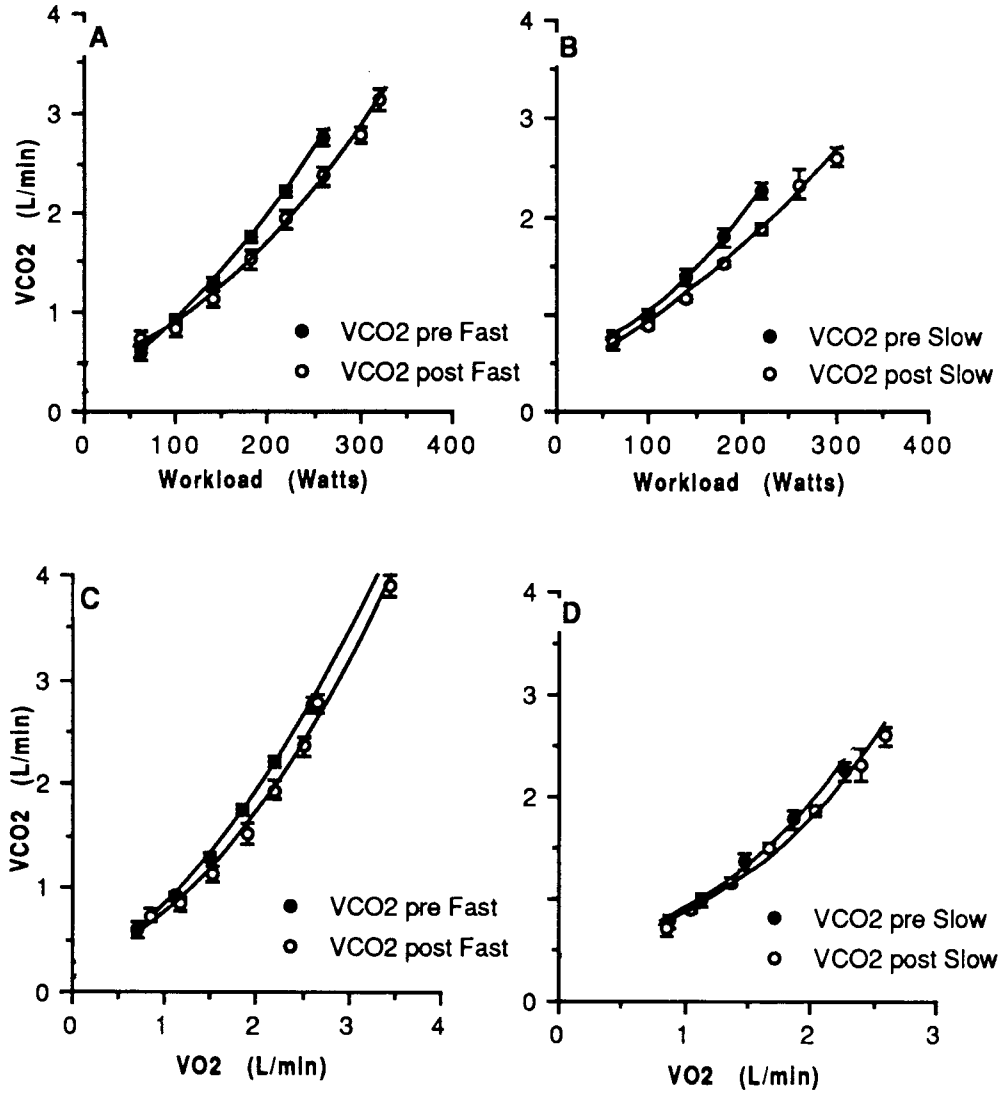


FIGURE 6.2. VCO_2 rises with increasing workload (panels A and B) and $V0_2$ (panels C and D) were best described by exponential rate equations before and after training ($p < 0.01$). VCO_2 was lower at workloads of 180-260W in the fast ramp ($p < 0.05$), and 100-220W in the slow ramp ($p < 0.05$) after training. Values shown are means ± S.E. for the 8 subjects.

Heart rate was significantly lower at all workloads after training ($p < 0.01$), with VO_2 only lower at workloads higher than 200W after training ($p < 0.05$). The best fit rate equations for these relationships are shown in table 6.3.

VCO_2 vs. work rate and VO_2

Fast ramp protocol. VCO_2 increased exponentially with increasing work rate (Figure 6.2 A) and with increasing VO_2(Figure 6.2 C). Differences in the linear vs. exponential equation R^2 VCO_2 vs work rate and VO_2 values are shown in table 6.1. VCO_2 was lower at workloads of 180W to 260W ($p < 0.05$), and VO_2's of 1.85 to 2.59 $l.min^{-1}$ after training ($p < 0.05$).

Slow ramp protocol. VCO_2 increased exponentially with increasing work rate (Figure 6.2 B) and with increasing VO_2 (Figure 6.2 D). Again, differences in the linear vs. exponential equation R^2 VCO_2 vs work rate and VO_2 values are shown in table 6.1. VCO_2 was lower at workloads of 100W to 220W ($p < 0.05$), and VO_2's of 1.06 to 2.27 $l.min^{-1}$ after training ($p < 0.05$). The best fit rate equations for these relationships are shown in table 6.3.

TABLE 6.1. COMPARISON OF R^2 VALUES OBTAINED IN FITTING DATA TO LINEAR REGRESSION EQUATIONS [L] AND EXPONENTIAL RATE EQUATIONS [E] BEFORE AND AFTER TRAINING.

	Fast Ramp R^2		Slow Ramp R^2	
	Before training	After training	Before training	After training
HR vs Workload				
(L)	0.981 ± 0.02	0.977 ± 0.006	0.952 ± 0.01	0.968 ± 0.01
(E)	0.977 ± 0.02	0.989 ± 0.004	0.981 ± 0.004*	0.994 ± 0..002*
VO_2 vs Workload				
(L)	0.975 ± 0.01	0.971 ± 0.01	0.967 ± 0.009	0.979 ± 0.005
(E)	0.985 ± 0.004	0.979 ± 0.006	0.989 ± 0.004*	0.994 ± 0..002**
VCO_2 vs VO_2				
(L)	0.950 ± 0.01	0.937 ± 0.01	0.903 ± 0.01	0.918 ± 0.01
(E)	0.993 ± 0.001**	0.990 ± 0.01†	0.928 ± 0.01**	0.937± 0..008*

Values shown are means ± S.E. of the 8 subjects. Differences between linear (L) and exponential (E) R^2 values before and after training are denoted as * = $p < 0.05$, ** = $p < 0.01$, and † = $p < 0.001$.

Respiratory exchange ratio (RER) vs. VO_2

RER was lower at VO_2's of 1.49 to 2.50 $l.min^{-1}$ in the fast ramp after training (Figure 6.3 A), and at VO_2's of 1.06 to 2.27 $l.min^{-1}$ in the slow ramp after training (Figure 6.3 B; $p < 0.01$).

Effect of training on blood [La^-] and V_I

VO_2 vs. blood [La^-]

Fast ramp protocol. The effects of training on the association between the rises in blood [La^-] with increasing VO_2 are illustrated in Figure 6.4 A. Blood [La^-] increased exponentially, and was lower at a VO_2's of 0.7 to 2.2 $l.min^{-1}$ after training ($p < 0.01$). VO_2's at dLa/dVO_2 slopes of 1, 2, and 3 were higher, indicating a slower rate of lactate accumulation during exercise after training (Table 6.2). An illustration of one subjects fast ramp blood "lactate slopes" is shown in Figure 6.5.

Slow ramp protocol. Figure 6.4 B shows the exponential increase in blood [La^-], and also the lower blood [La^-]'s at VO_2's of 0.85 to 2.27 $l.min^{-1}$ during exercise after training ($p < 0.01$). Again, VO_2's at dLa/dVO_2 slopes of 1, 2, and 3 were higher, indicating a slower rate of lactate accumulation after training (Table 6.2). The best fit rate equations for the VO_2 vs [La^-] relationships are shown in table 6.3.

V_I vs. VO_2

V_I increased exponentially in both the fast and slow ramp protocols before and after training. V_I was lower at VO_2's of 1.49 to 2.59 $l.min^{-1}$ during the fast ramp test after training (Figure 6.6 A; $p < 0.05$). During the slow ramp test, V_I was lower at VO_2's of 0.85 to 2.27 $l.min^{-1}$ after training (Figure 6.6 B; $p < 0.05$).

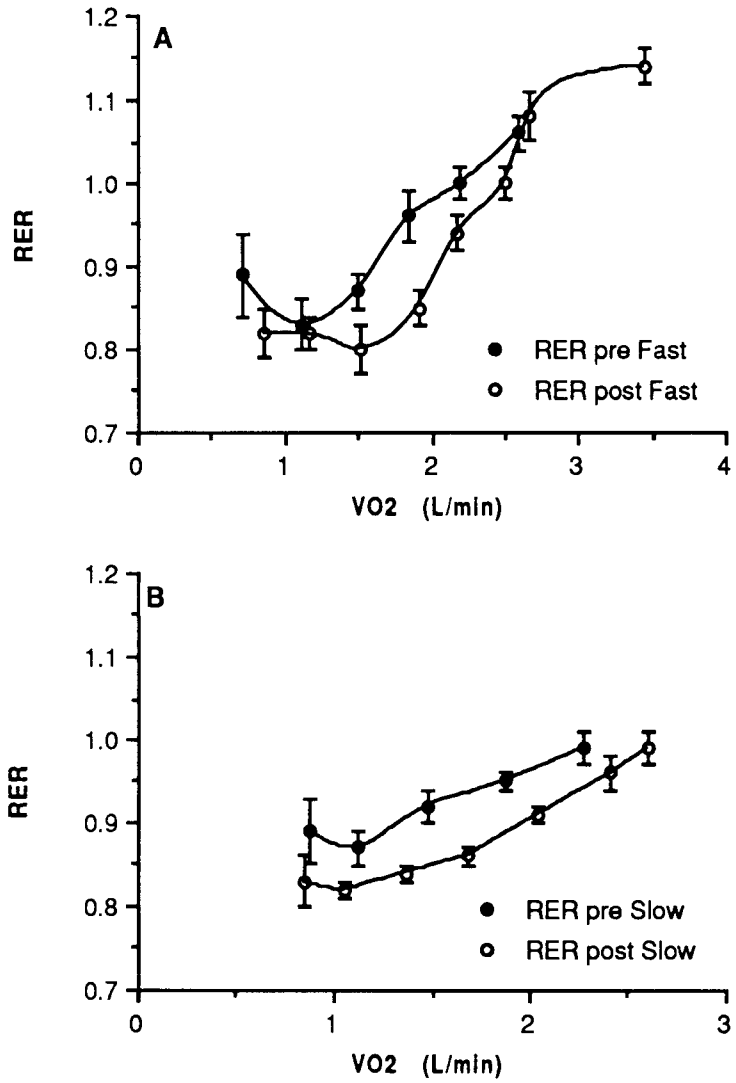


FIGURE 6.3. Endurance training resulted in lower RER values at VO_2's of 1.49 to 2.50 l.min^{-1} in the fast ramp (panel A; $p < 0.01$), and $V0_2$'s of 1.06 to 2.27 l.min^{-1} in the slow ramp (panel B; $p < 0.01$) after training. Values shown are means ± S.E. for the 8 subjects.

TABLE 6.2. INFLUENCE OF ENDURANCE TRAINING ON RISES IN BLOOD LACTATE AND MINUTE VENTILATION VOLUME WITH INCREASING OXYGEN CONSUMPTION.

	Fast Ramp		Slow Ramp	
	Before training	After training	Before training	After training
VO_2 at dLa/dVO_2				
slope 1	0.87 ± 0.29	1.93 ± 0.25*	1.12 ± 0.12	1.42 ± 0.10*
slope 2	1.61 ± 0.13	2.33 ± 0.25*	1.55 ± 0.10	1.82 ± 0..08*
slope 3	2.04 ± 0.13	2.55 ± 0.26**	1.81 ± 0.10	2.06 ± 0.08*
dV_I/dVO_2 at dLa/dVO_2 slopes of 1, 2, 3.				
slope 1	1.04 ± 0.02	1.10 ± 0.03 NS	1.07 ± 0.01	1.10 ± 0.01*
slope 2	1.09 ± 0.01	1.13 ± 0.04 NS	1.10 ± 0.01	1.13 ± 0.02*
slope 3	1.11 ± 0.01	1.14 ± .04 NS	1.11 ± .01	1.15 ± 0.02*

Values shown are mean VO_2's ± S.E. of the 8 subjects. Differences between slope values before and after training are denoted as NS = not significant, * = $p < 0.05$, and ** = $p < 0.01$. When the dLa/dVO_2 values were compared between ramp protocols, no differences in blood lactate accumulation rates were observed before training. However, VO_2's at dLa/dVO_2 slopes of 1 and 2 were significantly lower in the slow ramp after training ($p < 0.05$).

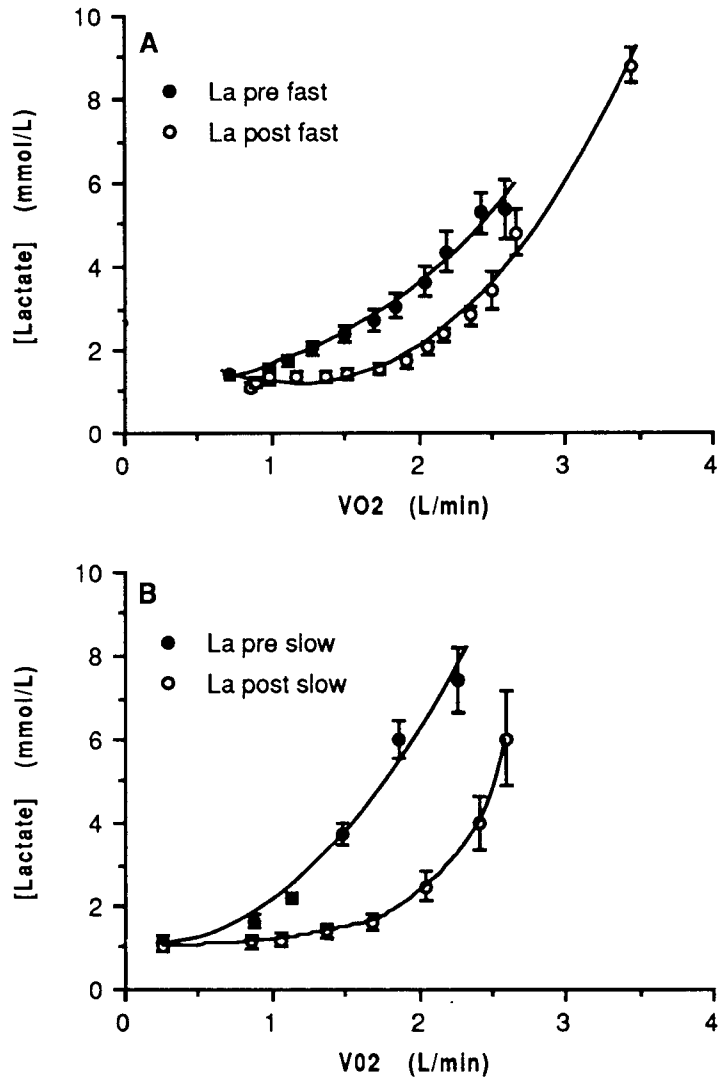


FIGURE 6.4. Endurance training markedly reduced the exponential rises in blood lactate accumulation of $V0_2$'s of 0.71 to 2.20 $l.min^{-1}$ in the fast ramp (panel A: $p < 0.01$), and $V0_2$'s of 0.85 to 2.27 $l.min^{-1}$ in the slow ramp (panel B; $p < 0.01$) after training. Values shown are means ± S.E. for the 8 subjects.

Calculations of dV_I/VO_2 showed that training increased the acceleration of ventilation at equivalent rates of blood lactate accumulation in the slow ramp test (Table 6.2). The best fit rate equations for these relationships are shown in table 6.3.

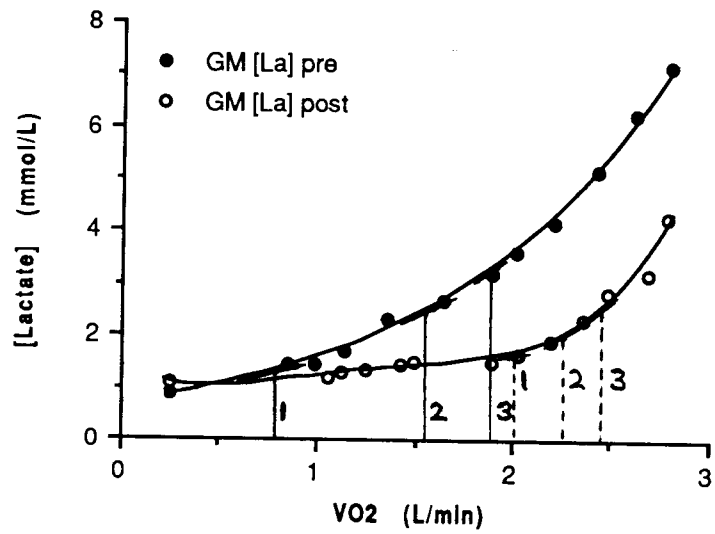


FIGURE 6.5. Fast ramp blood lactate data of one subject before and after training. Tangents drawn at the 1, 2, and 3 lactate slope VO_2's allow comparison of blood [La^-] vs $V0_2$ curves, without having to use a ruler to identify a threshold.

TABLE 6.3. EQUATIONS DESCRIBING RELATIONSHIPS BETWEEN THE METABOLIC RESPONSES IN FAST AND SLOW RAMP PROGRESSIVE EXERCISE.

	Before Training	After Training
FAST RAMP		
Workload vs. HR	y = 86.545 + 0.334x	y = 71.039 + 0.332x
Workload vs. VO_2	y = 0.169 + 0.009x	y = 0.282 + 0.009x
Workload vs. VCO_2	y = 0.415 * exp(0.001x)	y = 0.509 * exp(0.005x)
VO_2 vs. VCO_2	y = 0.36 * exp(0.154x)	y = 0.406 * exp(0.130x)
VO_2 vs. [La]	y = 7.497 * exp(0.212x) + (-7.33)	y = 0.620 * exp(0.604x) + (-0.08)
VO_2 vs. V_I	y = 15.374 * exp(0.116x)	y = 10.673 * exp(0.125x)
SLOW RAMP		
Workload vs. HR	y = 78.778 * exp(0.001x)	y = 71.91 * exp(0.003x)
Workload vs. VO_2	y = 0.623 * exp(0.006x)	y = 0.671 * exp(0.005x)
Workload vs. VCO_2	y = 0.517 * exp(0.001x)	y = 0.523 * exp(0.001x)
VO_2 vs. VCO_2	y = 0.415 * exp(0.144x)	y = 0.414 * exp(0.136x)
VO_2 vs. [La]	y = 1.416 * exp(0.794x) + (-0.87)	y = 0.008 * exp(2.48x) + (1.12)
VO_2 vs. V_I	y = 12.034 * exp(0.137x)	y = 11.287 * exp(0.135x)

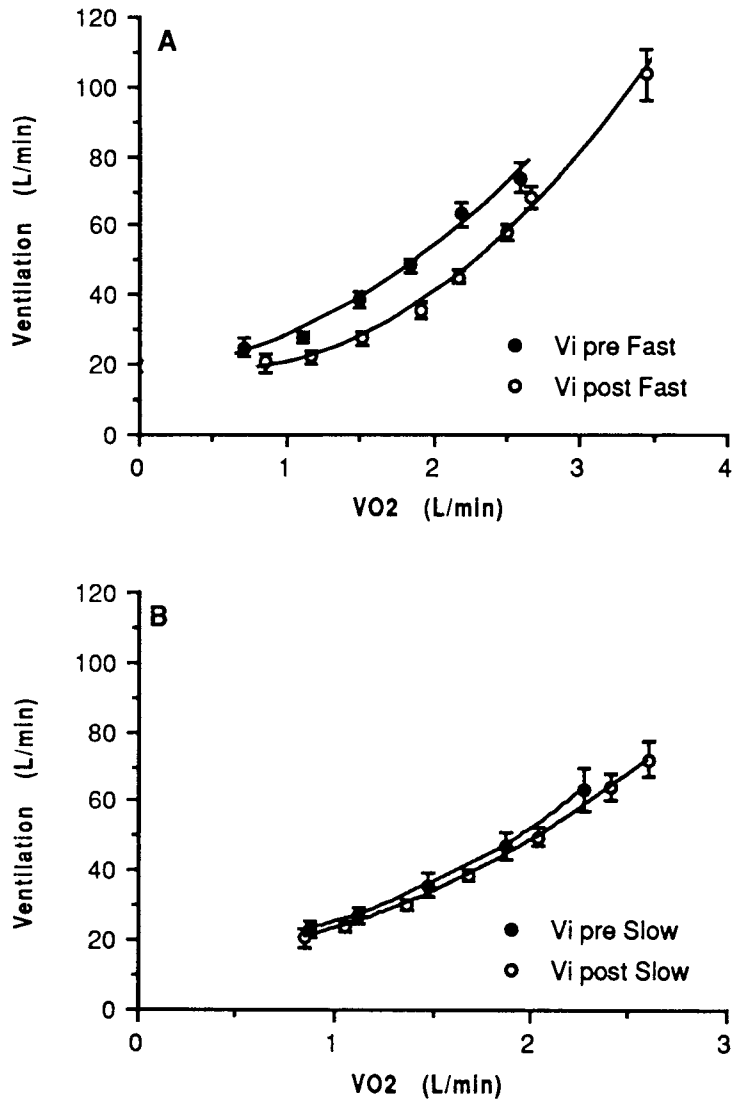


FIGURE 6.6. Endurance training reduced the exponential rises of V_I at $V0_2$'s of 1.49 to 2.59 $l.min^{-1}$ in the fast ramp (panel A; $p < 0.05$), and $V0_2$'s of 0.85 to 2.27 $l.min^{-1}$ in the slow ramp (panel B; $p < 0.05$) after training. Values shown are means ± S.E. for the 8 subjects.

Discussion of Training Effects on Respiratory Responses During Exercise

This study showed the well-documented phenomenon of linear increases in heart rate and VO_2 with increasing work rate (Noakes, 1988), in the fast exercise ramp before and after training (Figure 6.1 A and 6.1 C). However, with increasing work rate in the slow exercise ramp, both heart rate and VO_2 increased exponentially before and after training. The rise in heart rate suggests the possibility of "cardiovascular drift" during the slow exercise ramp, possibly due to a progressive drop in stroke volume, arising from vasodilation and a fall in diastolic pressure as heat accumulates. This effect could possibly be mediated by circulating catecholamines exerting their effects over the longer time in the slow exercise ramp compared to the fast exercise ramp.

The curvilinear rise in VO_2 is, however, difficult to explain. For VO_2 to rise as an exponential function of work rate, progressively more and more of the chemical energy from fuel oxidation has to be converted to kinetic energy (i.e., heat). It is possible that the longer work stages in the slow exercise ramp tests caused greater rises in muscle temperature. Brooks and Fahey (1985) have proposed that heat uncouples oxidative phosphorylation (ATP synthesis) from electron transport chain activity (O_2 consumption). It has been observed that 15 mins of exercise at around 90-95% VO_2max (personal communication from T. D. Noakes), and sustained exercise above the "anaerobic threshold" (Whipp, & Wasserman, 1972), results in a continual upward drift in VO_2 with no attainment of steady state . This finding is not dissimilar to the VO_2 response in this study, and may result from an increase in muscle temperature. In addition, higher muscle temperatures might also accelerate ATP wastage by the fructose-6-phosphate and fructose-1-6-diphosphate cycle in type IIB motor units.

One of the most dramatic responses to a programme of endurance training, is a marked attenuation in the rise of circulating blood [La^-] with increasing VO_2. This response was seen in this study at both the fast and slow exercise ramps after training (Figure 6.4 A and B). This attenuation in the rate of lactate accumulation in both exercise ramps was such that, the VO_2's at dLa/dVO_2 slopes of 1, 2, and 3 were all substantially increased after training (Table 6.2). The diminished blood [La^-]'s are likely due to reduced rates of carbohydrate oxidation, as evidenced in the lower RER's in both exercise ramps after training (Figure 6.3). The lower blood [La^-]'s observed in trained humans performing submaximal exercise, has been attributed to decreases in rates of lactate production (Favier, et al., 1986) or improvements in rates of lactate disappearance (Stanley, et al., 1985). The increases in skeletal muscle capillarity, mitochondrial density, and oxidative enzyme concentrations as a result of endurance training, could contribute to the reduced blood [La^-]'s observed in this study (Holloszy, et al., 1984). Furthermore, the lower blood [La^-]'s could also be due to increases in lactate removal via oxidation (Gertz, et al., 1981; Mazzeo, et al., 1986; Stanley, et al., 1988) and gluconeogenesis (Donovan, et al., 1990b). As shown in a previous section of this thesis, the reduced blood [La^-]'s are the combined result of a reduced rate of lactate appearance at exercise intensities requiring < 60% VO_2max, and an elevated rate of lactate metabolic clearance at exercise intensities up to 75% VO_2max after training.

The rate of increase of work rate markedly influences the blood lactate concentration at different VO_2's. It has been shown that blood [La^-] increases more markedly for a given VO_2 during slow ramp increments than during fast ramp increments in work rate, due to the longer duration at each work rate (Hughson, et al., 1982). Determination of the VO_2's at dLa/dVO_2 slopes of 1, 2 and 3 in this study, showed that the exercise ramp had no effect on the rate of lactate accumulation before training (Table 6.2). For instance, the VO_2 at dLa/dVO_2 slopes of 2, was 1.61 ± 0.13 in the fast, compared to 1.55 ± 0.10 l. min^{-1} in the

slow exercise ramp. However, at this same blood lactate slope of 2 after training, the VO_2 in the slow exercise ramp (VO_2 of 1.82 ± 0.08 l.min^{-1}), was lower than in the the fast exercise ramp (2.33 ± 0.25 l.min^{-1}). The tendency for blood lactate to accumulate at lower VO_2's in the slow exercise ramp was seen only at the dLa/dVO_2 slopes of 1 and 2. The VO_2's at a dLa/dVO_2 slope of 3 were similar in both exercise ramps after training. Together, these observations suggest that endurance training may improve the clearance of lactate from the muscle into the blood. Support for this observation is presented in Chapter 4 where it was shown that the rate of lactate clearance was higher after training.

Minute ventilation was reduced at equivalent metabolic rates in both exercise ramps after training (Figure 6.5 A and B). This adaptation to training during submaximal exercise has been consistently observed in studies of athletes (Byrne-Quinn, Weil, Sodal, Filley, & Grover, 1971; Miyamura, Yamashina, & Honda, 1976), and is most pronounced in endurance athletes (Martin, Sparks, Willich, & Weil, 1979). It has been proposed that the additional rise in ventilation with increasing work rate above the "anaerobic" threshold is a linear function of lactate concentration (Hughes, et al., 1968; Sutton, et al., 1979), the so called humoral drive hypothesis advanced by Wasserman et al. (1973). The results of this study contradict this hypothesis. In this study, endurance training dissociated the exponential rise in blood [La^-] during incremental exercise from the continuous increase in ventilation volume (Table 6.2). Calculations of dV_I/dVO_2 at equivalent rates of blood lactate concentration showed that endurance training shifts the relationship between minute ventilation volume and blood [La^-] to the left. Whereas dV_I/dVO_2 values were unaffected by training, the lower lactate slope VO_2's were markedly increased in the slow exercise ramp. Thus, ventilation increased with little change in blood [La^-] at the lower VO_2's, and at the higher VO_2's, blood [La^-] increased with little rise in minute ventilation.

Increases in ventilation during exercise may result from nonhumoral stimuli originating in exercising muscle or the brain, thereby having a neurogenic origin (Hagberg, et al., 1982; Whipp, 1981). Lactic acidosis is but one of a number of factors influencing pulmonary ventilation (Jones, et al., 1982; Whipp, 1981), but has been highly publicized by Wasserman et al. (1964; 1973; 1975) as the stimulus for increased ventilation during incremental exercise. These investigators have also proposed that increases in venous CO_2 load as a result of bicarbonate buffering of lactate (i.e., $H^+ + HCO_3^- <---> H_2CO_3 <---> H_2O + CO_2$), is implicated in the control of ventilation.

The results of this investigation challenge the traditional belief in exercise physiology that the respiratory compensation for metabolic acidosis with increasing VCO_2, is due to a sudden onset of blood lactate accumulation. Gevers has shown (1977) that when ATP synthesis is taken into consideration and the likely charges at cytosolic pH are summed, the breakdown of glucose or glycogen to lactate does not produce a large net gain of protons (Eq. 1):

(1) glucose + 2 $MgADP^-$ + 2 P_i^{2-} -----> 2 $lactate^-$ + 2 $MgATP^{2-}$

Instead, protons arise from glycolytic ATP turnover and are therefore generated irrespective of whether lactate is formed or pyruvate is delivered to the mitochondria for oxidation (Eq. 2):

(2) $Mg\,ATP^{2-}$ ---------> $MgADP^{-} + P_i^{2-}$ ----------> H^+

pyruvate or lactate <---------- glycolysis <---------- glucose or glycogen

Since any increase in carbohydrate metabolism would be expected to accelerate proton formation, we suggest that blood lactate accumulation is more a result of, than a cause of metabolic acidosis. It is our contention that, when the capacity of the muscle to exchange intracellular protons for extracellular Na^+ is exceeded, increases in cytosolic $[H^+]$ shift the lactate dehydrogenase and lactate translocase equilibria towards lactate production and efflux (Eqs. 3 & 4):

(3) $pyruvate^- + NADH + H^+ \rightleftharpoons lactate^- + NAD^+$

(4) $H^+ + lactate^-$ (in) $\rightleftharpoons$ $H^+ + lactate^-$ (out)

Thus, the theoretical expectation is that the exponential rises in ventilation and blood lactate concentration with increasing VO_2 are independent consequences of the acceleration of carbohydrate oxidation during progressive exercise.

The results of this study do not support the CO_2 flow/metabolic acidosis hypothesis for the observed increase in ventilation during incremental exercise. Carbon dioxide production was lower in both exercise ramp tests after training (Figure 6.2), yet minute ventilation was significantly higher at equivalent rates of blood lactate accumulation (Table 6.2). This finding was evident at blood $[La^-]$'s as low as 1 mM. Since the ventilatory response was clearly elevated even at low $[La^-]$'s after training, we suggest that the contribution of $[H^+]$

and CO_2 flow to the stimulation of ventilation at lower intensities of work in the trained condition is attenuated. Dissociation between changes in blood [La^-] and changes in V_I have also been demonstrated in patient's with McArdle's disease (Hagberg, et al., 1982), and in carbohydrate depleted individuals (Heigenhauser, et al., 1983; Hughes, et al., 1982).

We propose that training, through feedback or feedforward mechanisms, may stimulate increases in ventilatory drive during exercise, even at low workloads. The existence of both afferent (Kao, 1963; McCloskey, & Mitchell, 1972; Sargeant, Rouleau, Sutton, & Jones, 1978), and efferent neural input (Eldridge, Millhorn, & Waldrop, 1981) to the respiratory control center during exercise has been clearly demonstrated. Ascending neural stimuli from working muscle have received much attention in this regard. However, it has been shown that spinal cord transection does not block the observed hyperpnea when hindlimb muscles are electrically stimulated (Dempsey, Vidruk, & Mastenbrook, 1980). Evidence to support the concept of a descending neural stimulus to hyperpnea has been tested experimentally using the thalamic cat model (DiMarco, Romaniuk, Euler, & Yamamoto, 1983; Eldridge, et al., 1981; Shik, Severin, & Orlovskii, 1966). These demonstrations of a locomotor-hyperpneic coupling have been criticized as to whether the events demonstrated are accurate representations of what actually occurs during physiological exercise (Dempsey, Vidruk, & Mitchell, 1985). The evidence from this investigation tends to suggest the primacy of a neural component rather than a humoral component for the ventilatory response observed following training.

In summary, blood lactate concentration increased exponentially during exercise before and after training. Accelerations of V_I (dV_I/dVO_2) at the 1, 2, and 3 "lactate slopes", showed that training dissociated the continuous increase in ventilation volume from the exponential rise in blood lactate concentration.

CHAPTER 7

Summary and Conclusions

When compared with untrained individuals during submaximal exercise, diminished blood lactate concentrations are seen in trained athletes and in subjects who have undergone endurance training. The main purpose of this study was to determine why endurance training results in a decreased blood lactate concentration during submaximal exercise. In addition, the metabolic fate of lactate produced during exercise and the effects of training on respiratory responses during different ramp exercise protocols were determined.

Irrespective of the absolute numbers, it is clear that the lower blood lactate concentrations during progressive exercise after training are due to a combination of effects. At low absolute work rates, lactate rates of appearance are diminished, while rates of lactate clearance are unchanged. At higher absolute work rates, lactate rates of appearance are similar and, lactate rates of clearance are sustained at high levels (Figures 4.2 A & 4.3 A). During exercise at the same absolute work rates after training, the subjects were exercising at a lower relative percentage of VO_2max. Here, the diminished lactate concentration after training was due to a reduced lactate Ra up to 60% VO_2max (Table 4.2). At work rates above this exercise intensity, lactate Rd was increased after training (Table 4.2). Lactate MCR was sustained at high levels at all relative exercise intensities up to 75% VO_2max after training (Table 4.2).

Therefore, during competitive endurance events where athletes perform sustained exercise at intensities around 80-90% VO_2max, lactate production is probably greater than in less well-conditioned individuals. This can be inferred from the similar lactate Ra's at test

termination, which occurred at 88% VO_2max before and 75% VO_2max after training. Thus, the reduced blood lactate concentration seen at high power outputs during exercise after training, is mainly due to high rates of lactate removal, likely via the lactate shuttle.

When the removal of lactate by oxidation or gluconeogenesis was examined during exercise, it was determined that the improved removal of lactate after training at equivalent venous lactate concentrations (Figure 4.3 B), appears to be due to increases in oxidative clearance (Figures 5.1 B & 5.2 B), and not due to increased rates of gluconeogenesis. Estimated rates of lactate removal via gluconeogenesis increased during exercise both before and after training (Figure 5.4 A & B). However, both lactate oxidative clearance and lactate removal via gluconeogenesis during exercise, were reduced at equivalent metabolic rates after training (Figures 5.2 A & 5.4 A). Furthermore, the estimated rates of gluconeogenesis from rising lactate concentrations during progressive exercise were much higher than has previously been reported in prolonged steady state exercise studies where blood lactate levels remain low.

Studies of the respiratory compensation for the metabolic acidosis during progressive exercise showed that whereas the rate of blood lactate concentration is slowed during submaximal exercise following endurance training (Figure 6.4), the rise in ventilation volume is largely unaltered (Table 6.2). The study also confirmed previous observations, that blood [La^-] increases as an exponential function of increasing oxygen uptake during ramp exercise tests. This result was seen both in exercise ramps of differing work rate and time increments (Figure 6.4 A & Table 6.3). Finally, the study showed that training dissociates the continuous increase in ventilation volume from the exponential rise in blood lactate concentration.

Recommendations for Future Research

Changes in the concentration of blood lactate during exercise cannot be solely attributed to increased rates of lactate production. In this study, the rate of lactate appearance was only diminished at exercise intensities eliciting < 60% VO_2max after training, while the clearance of lactate was maintained at higher levels after compared with before training. As reviewed previously, the blood lactate concentrations observed during exercise are dependent on the balance between release and uptake of lactate from diverse tissues. Alterations in the intensity of exercise performed by an individual, can cause tissues to shift from lactate producers to lactate consumers, or vice versa.

To what extent skeletal muscle, the heart, liver, skin and other tissues affect blood lactate accumulation during exercise remains to be determined. Once the methodology to make these determinations is in place, quantification of these tissues' role in lactate production and/or removal, and the relative importance of each tissue in this role will be achieved.

A major effector of lactate production is epinephrine, through its modulation of muscle glycogenolysis and glycolysis. The lower blood lactate concentrations observed in trained individuals compared to untrained individuals during submaximal exercise, are partly due to a decreased effect of epinephrine on muscle glycogenolysis. How epinephrine affects lactate production during prolonged low (< 60% VO_2max) and high (> 85% VO_2max) intensity steady state exercise is not yet known. Furthermore, the role of lactate as an energy source during prolonged submaximal exercise, either as an oxidizable substrate, gluconeogenic precursor or both, is not known. It may be that lactate contributes significantly to overall carbohydrate oxidation during prolonged submaximal exercise.

The factors which determine the rate at which decreases in lactate concentration occur after training in humans have yet to be identified. After a week of endurance training, using a training protocol similar to that employed in this study, Gaesser and Poole (1986) showed a decrease in lactate concentration during submaximal exercise. Winder et al. (1979) showed decreases in plasma catecholamine concentrations during submaximal exercise after three weeks of endurance training. It needs to be determined if this reduced catecholamine response contributes to the rapid change in lactate concentration during submaximal exercise after training. In view of the possible effects of epinephrine on lactate appearance, a study incorporating Beta 2 blockade to determine the effects of training on lactate kinetics would be useful. In addition, it also remains to be established if lactate clearance in humans is improved during the early stages of endurance training.

APPENDIX A : Assay Methods

Blood lactate concentration measurements

This method was adapted from Gutmann and Wahlefeld (1974).

Collection of the sample

Plastic Greiner test-tubes containing 2 ml of 0.6N perchloric acid (PCA) were weighed on a Sartorius 1412 MP8-1 scale (Gottingen, West Germany) and the weights recorded. These tubes were kept in a refrigerator until the samples were ready to be collected, at which time the tubes were kept cold on ice. Approximately 1 ml of whole blood sample was added to the tube containing the PCA, shaken well, and then reweighed to calculate the actual volume of blood added. The tubes containing the sample were kept on ice for 10 minutes, and then cold centrifuged for 15 minutes at 2000 rpm. The supernatant was then decanted into labelled Eppendorf test-tubes, and stored at -20° C until the assay was performed.

Assay method

Glass test-tubes were labelled as follows; 3 x blanks, 2 x standards, and then sample tubes in duplicate. Reagents were added to each of the tubes as follows;

	Standard	Blank	Test
0.4M Hydrazine/0.5M glycine buffer, pH 9.0	1.0 ml	1.0 ml	1.0 ml
NAD, 30mM	0.1 ml	0.1 ml	0.1 ml
PCA, 0.6M	-	0.1 ml	-
LDH (5mg protein/ml)	0.01 ml	0.01 ml	0.01 ml
Supernatant	-	-	0.1 ml
Lactate standard (dilution of 1.0N)	0.1 ml	-	-
H_2O	-	0.1 ml	-

The hydrazine /glycine buffer was 0.4 M hydrazine and 0.5 M glycine in distilled H_2O, adjusted to pH 9.0. The NAD solution (± 30 mM) was prepared by dissolving 100 mg NAD in 5 ml distilled H_2O, and then stored at -20° C until needed. The LDH suspension (± 5 mg protein/ml) was stored at 4°C and used undiluted. A dilution of 1.0M lactate standard was used.

The standard, blank and test solutions were separately mixed on a vortex mixer. These solutions were covered with parafilm, and then placed in an incubator to react for 30 minutes at 37° C. Optical density changes were recorded with a Beckman DU-62 spectrophotometer (Beckman Instruments Inc., England) previously zeroed at 340 nm against distilled H_2O. The following calculation was used to determine the lactate concentration of the samples in mM. l^{-1}:

$$\frac{\Delta OD_{340}}{6.22} \times \frac{1.31}{0.1}$$

Plasma glucose concentration measurements

For glucose determinations 2 ml of blood were collected in Vac-U-Test tubes (Radem Lab Equipment, Sandton, S.A.) containing potassium oxalate and sodium fluoride. The samples were cold-centrifuged for 10 minutes at 2000 rpm. The plasma was then stored at -4° C until glucose was assayed. Glucose assays were performed in duplicate on a Technicon RA-XT (Technicon, Ireland) automated glucose analyzer. The determination of plasma glucose concentration by the analyzer is based on the glucose oxidase method (Hyvarinen, & Nikkila, 1962) and was checked by running a standard after every 10 samples.

Plasma FFA concentration measurements

This assay was modified and adapted from Devic-Mikac et al. (1973).

2 ml of blood was collected in SST separation tubes (Radem Lab Equipment, Sandton, S.A.). The sample was centrifuged for 10 minutes at 2000 rpm. The plasma was then stored at -4° C until FFA concentrations were assayed.

Following the preparation of the standard solutions in triplicate, 500 ul of 5% NaCl was pipetted into each sample tube (10x 100 mm new borosilicate glass test-tubes). To this was added 500 ul of a Cu-TEA solution (equal volumes of 1 M TEA and 6.45% $Cu[NO_3]_2$) freshly made up for each assay. Duplicate 100 ul samples and triplicate standards were then added to each tube. All tubes were vigorously shaken clockwise and counter-clockwise to ensure adequate mixing. 2 ml of $CHCl_3$ was added to each test-tube. All test-tubes were once again vigorously shaken as previously described. The aqueous phase (upper) in each

sample was aspirated and discarded. 100 ul of colour reagent per sample [1,5-diphenylcarbohydrazide, 1.5% (w/v) in acetone] was then made up. 200 ul of the lower phase of each sample was pipetted into a clean test-tube (10x 75 mm new borosilicate glass), to which was added 1 ml $CHCl_3$ and 100 ul of the diluted colour reagent. All samples were mixed again, and then allowed to stand for 15 minutes to allow the colour development to take place. The samples were then read at 550 nm in a Beckman DU-62 spectrophotometer (Beckman Instruments Inc., England). A standard curve was established and the FFA concentration of each sample was expressed in $mM.l^{-1}$. Stock standard (Palmitic acid, MW 256.4, Sigma) was made up to 2.0 $mmol.l^{-1}$ in redistilled chloroform ($CHCl_3$, A.R.), and serial dilutions were made to obtain concentrations of 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0
$mmol.l^{-1}$.

Plasma glucagon concentration measurements

This method is adapted from Fourie (1983).

Plasma was separated from 3 ml of blood, which had been collected in Vac-U-Test-tubes containing 0.2 ml Trasylol in lithium heparin (14.3 U.S.P. Units ml/blood), by centrifugation at 2000 rpm for 10 minutes. The samples were stored at -4° C until the assay was performed.

Measurement of the sample.

(i) Incubation.

All assays were performed in plastic cuvettes (11x 52 mm, LKB, Sweden). Total extracts were assayed in duplicate, and gel filtrates described later were measured singly. The

standard curve was obtained from triplicate tubes containing 5, 10, 20, 50, 100, 200, 500, 1000 pg of 3x purified crystallized porcine glucagon (Lilly, USA), made up to 800 ul with diluent [Glycine/NaCl buffer - see below; 0.25% albumen (B.S.A.) and 1% normal rabbit serum]. In addition, tubes containing samples and standards without any of the V_{13} antibody, described later, were included in the assay to determine the non-specific binding to charcoal. The total counts of ^{125}I -glucagon, usually between 7000-8000 cpm, were measured in tubes containing only tracer and diluent. To determine zero binding (Bo) a set of tubes were included containing no standard.

To all samples, standards, and Bo tubes were added 100 ul of antibody (V_{13}, raised in the Dept. of General Surgery, University of Cape Town Medical School) and 100 ul of tracer. The tubes were mixed between each addition. The final volume in each set of tubes was adjusted to a final volume of 1 ml with distilled H_2O and the tubes were then incubated for 72 hours at 4° C.

(ii) Charcoal separation.

The unbound ^{125}I -glucagon was absorbed onto 500 ul dextran coated charcoal in glycine buffer (0.2 M glycine, pH 8.8; 0.1 M NaCl, and 500 KIU/ml Trasylol). 100 ul inactivated horse serum was added to standards and plasma free samples. The tubes were mixed, incubated at 4° C for 45 minutes and then centrifuged at 4° C for 15 minutes at 3000 rpm. The supernatant was discarded and the charcoal pellets were placed in a gamma scintillation counter (Packard Instrument Co., Downers Grove, Illinois) for 10 minutes or until 10 000 disintigrations had been counted.

(iii) Calculation.

After subtraction of the counts produced by non-specific binding, the level of unbound ^{125}I -glucagon was calculated as a percentage of the total counts.min^{-1} in each sample and standard. To calculate the antibody-bound labelled glucagon these values were subtracted from 100%. The % values obtained in this way for Bo (zero standard) tubes were given a value of 100% (Bo = 100% binding of the label to antibody and is therefore zero for the binding of the standard to the antibody).

All other % bound (B) values of standards and samples were expressed as a % of this (B/Bo x 100). Standard curves were constructed by plotting the B/Bo x 100 values against the concentration of standard (picograms). Results were expressed as pg.ml^{-1}. The immuno reactive glucagon content (IRG) of the unknowns was obtained by reading the corresponding B/Bo x 100 off the standard curve.

(iv) Procedure for the iodination of glucagon with 125nI.

Transfer of ^{125}I from ^{125}Na-I to the tyrosine residues on glucagon involved a step by step addition of the following reagents;

5 - 7.5 ul, 1 mg.ml^{-1} glucagon freshly weighed. pH 2.3
25 ul 0.5 M phosphate buffer. pH 7.5
10 ul Na^{125}I (Weill Organisation)
5 ul 0.4% Chloramine T in 0.05 M of phosphate buffer

This completes the oxidation of ^{125}Na-I and the ^{125}I transfers to tyrosine residues on the glucagon molecule. After a brief mixing, the reaction was stopped with 20 ul 0.24%

sodium metabisulphite in 0.05 M phosphate buffer. At this point 50 ul of 1% potassium iodide was also added to dilute the ^{125}I still unattached.

Separation of the labelled hormone from the reaction mixture was carried out by passing the mixture through a plastic column 0.7 x 30 cm (Biorad), containing Sephadex QAE 25. Immediately before application to the column, 10 ul of 1M tris followed by 1 ml of the elution solution was added to the ^{125}I reaction mixture. Equilibration of the Sephadex gel and elution were carried out with a 0.08 M tris buffer containing 0.08 M NaCl, 0.02 M HCl, 1% human albumen and 500 KIU.ml^{-1} Trasylol, pH 8.6.

The flow rate of the column was adjusted to 10 ml.hr^{-1} using a peristaltic pump (Pharmicia P3). Fractions of 2 ml were collected and the radioactivity in each fraction counted. A graph was plotted of cps against the fraction number and the fractions with the highest cps were combined and stored in 500 ul aliquots at -20° C. Glucagon was always labeled within a week prior to the experiment.

To determine binding of ^{125}I to glucagon, a few drops of the fractions with the highest cps were applied to cellulose acetate paper strips soaked in Veranol buffer, pH 8.6, and subjected to high voltage electrophoresis. Any free ^{125}I moves with the front leaving the labelled glucagon at the starting point. This method however, gives no indication of the binding of the label to the V_{13} antibody and so a standard curve was run to determine the quality of the label. V_{13} was able to recognize 5 pg of pancreatic glucagon with 97% confidence. At ID50 (Inhibitory Dose), the concentration of pancreatic glucagon required to displace 50% of tracer was 36 pg.ml^{-1} which is comparable to the 35 pg.ml^{-1} value for the well characterised and widely used Ungers 30K antibody.

Plasma insulin concentration measurements

Plasma was separated from 3 ml of blood, which had been collected in Vac-U-Test-tubes containing 0.2 ml Trasylol in lithium heparin (14.3 U.S.P. Units ml/blood) by centrifugation at 2000 rpm for 10 minutes, and then stored at -4° C pending analysis. A Phadeseph Insulin RIA package (Pharmacia Diagnostics AB, Uppsala, Sweden) was used to determine plasma insulin concentration in duplicate.

Muscle glycogen determination

(i) Preparation of the muscle biopsy sample for glycogen assay

Frozen (-196°C) muscle biopsy samples (10-20mg) were added to weighed Eppendorf tubes (Greiner) containing 200 ul of cold 40% w/v KOH. Tubes were then re-weighed to determine muscle weight and heated to 95°C for 30 minutes to dissolve the tissue. When the tubes had cooled, 0.8ml of absolute ethanol was added and the samples were refrigerated overnight to allow time for the glycogen to precipitate.

On the next day, the tubes were spun for 2 minutes in an Eppendorf 5413 centrifuge (Hinz, West Germany), and the supernatant was aspirated. The pellet was then washed three times with cold absolute ethanol and each time respun for 2 minutes. The final pellet was then acidified with 0.2 ml of 2N HCl and heated for 3 hours to 95°C to hydrolyze the glycogen to glucose.

On the following day the samples were neutralized to pH 7,5 - 7,7 with approximately 0.3 ml of 2N NaOH and reweighed to determine the final volume.

(ii) Measurement of glucose

Glucose was analyzed according to the enzymatic method in Bergmeyer (1974) shown below:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} 6\text{ phosphogluconate} + \text{NADPH} + \text{H}$$

In this assay the hexose equivalents of glycogen present were measured by the increase in NADPH absorbance at 340 nm. The composition of the reaction mixture was as follows:

	Reaction mixture per cuvette
Tris buffer, pH 7.5 (0.2M)	1.0 ml
$MgCl_2$ (1M)	0.1 ml
ATP (10mM)	0.2 ml
NADP (15mM)	0.1 ml
Distilled H_2O	1.4 ml
TOTAL	**2.8 ml**

To each 2.8 ml of reaction mixture was added 0.2 ml of either sample, 1.0 mM glucose standard or distilled water. Samples were assayed in duplicate and standards and blanks were assayed in triplicate. For the assays the spectrophotometer (Beckman DU-62, Beckman Instruments, England) was first zeroed with a cuvette containing distilled water, and then the initial absorbance of each sample was read at 340 nm. Commercial glucose-6-

phosphate dehydrogenase (0.01 ml) and hexokinase (0.01 ml) were then added to each sample and the decrease in optical density (Δ OD) was monitored until there was no further change. The glycogen content of the sample was then calculated as follows:

$$\frac{\Delta \text{ O.D. (sample)}}{\Delta \text{ O.D. (std)}} \times \frac{\text{final extraction volume (ml)}}{\text{muscle weight (g)}} = \text{mmol.kg}^{-1} \text{ wet weight}$$

Myosin ATP-ase assay method for muscle fibre typing

(i) Slide Preparation

The muscle biopsy sample was placed flat on a glass slide in an ice-bucket for 30 minutes. The muscle specimen was trimmed, orientated on cork and embed in OCT compound (Tissue Tek #4583, Miles Scientific, Naperville, Illinois). The n-Pentane was prepared, placed in a beaker, and suspended in a container of liquid N_2. When the n-Pentane was almost frozen through, leaving a small unfrozen well in the centre, the cork was immersed in the cold pentane with embedded tissue and agitated rapidly for ± 4 seconds. At this stage the frozen tissue was wrapped in parafilm, placed in a plastic bag and stored overnight at -20° C. When ready for sectioning, the cork with the imbedded tissue specimen was attached to the chuck of a SLEE-HRM cryostat (South London Electrical Equipment Co., London, England) using liquid N_2 and OCT compound. Sections of sample 10 u thick were cut, attached to glass slides and allowed to dry in the air for not more than 30 minutes. All glass slides were then coated with 0.1% poly-l-lysine and left to dry.

Buffers were then made up as follows. The buffer for pH 4.2 medium consisted of barbitol (1.94 g% sodium acetate and 2.94 g% sodium barbitone), and 0.1M HCl made up in 100

ml distilled water, with the pH being adjusted to 4.2 with barbitol buffer and 0.1M HCl. The buffer for pH 9.4 medium with ATP substrate consisted of 2% sodium barbitone, 2% $CaCl_2$ and 0.038g of ATP, with the pH being adjusted to 9.4 with 0.1M NaOH and 0.1N HCl. Slides for pH 4.2 were incubated with the buffer at 37° C for 5 minutes. Following this incubation they were removed and placed together with an unincubated slide into pH 9.4 substrate for 20 seconds. The slides were then rinsed 3 times with 1% $CaCl_2$ for approximately 2 minutes. Following this the slides were rinsed 2 times with 2% $CoCl_2$ for approximately 5 minutes. All slides were washed thoroughly with distilled water and then immersed in ammonium sulphide and rinsed well. The nuclei were then stained with haematoxylin and methylene in water. The slides were then dehydrated, cleared and mounted. The slides were immediately photographed with an Olympus PM6 camera (Olympus, Tokyo, Japan) attached to an Olympus-BH2 photomicroscope. All slides were photographed onto Kodak black/white ASA 125 22DIN 35 mm film, at 10 x 4 x 3.3 magnification. Following development of the film, muscle fibres were counted according to the staining patterns using an IBAS 1 interactive digitizing image analysis system (Kontron, West Germany). Muscle fibre types were identified as Type 1, which stained dark, and Type 2, which stained grey/shaded after exposure to a pH of 4.2. A minimum of 100 fibres per type per specimen were counted.

(ii) Principle of ATPase staining

The histochemical detection of myosin ATPase involves a complex series of reactions. First, the tissue section is incubated in an alkaline solution containing ATP and calcium so that the phosphate released by myosin ATPase precipitates as insoluble calcium phosphate at the site of enzyme activity. Next, the section is exposed to cobalt chloride and the cobalt is exchanged for the calcium in the calcium phosphate. Finally, the section is exposed to

ammonium sulphide which results in the formation of a black insoluble, cobaltous sulphide which can be visualized and quantitated.

Determination of muscle fibre diameter

Prints of the muscle fibre types were also used to measure changes in fibre diameter with training. Diameters were digitized using an Ibas 1 computer assisted Interactive Image Analysis system (Kontron, West Germany). Again, a minimum of 100 fibres per fibre type for each subject were measured.

APPENDIX B

Summary of results given to subjects

20 May 1989

Dear Participant

Congratulations on completing this exercise program and the testing that accompanied it! The information which is supplied to you in this report is confidential in that no one else has had access to it. You may however show it to whom you wish. Some of the information will be familiar to you, having been given to you during the early stages of the project. I believe that the new information will be of interest, and also I trust, of use to you. I have not included all the results of the project in this summary - a large portion of those results are quite technical in nature, and would probably not be of interest or use to you.

The data summarized here is divided into a cardiovascular section, body composition, and into a muscle section. I have included the grouped data so that you can compare your performance to that of your peers.

CARDIOVASCULAR PERFORMANCE.

The ability to supply energy for activities lasting more than 90 seconds depends on the consumption and utilization of oxygen. Most of the physical activities in daily life demand time periods longer than 90 seconds; the consumption of oxygen therefore provides the energetic basis of our existence. Oxygen consumption increases from rest, to easy, to difficult and maximal workloads. The maximum rate at which an individual can consume oxygen (VO_2max) is an important determinant of the peak power output and of the maximal sustained power or physical work capacity of which an individual is capable. The capacity for VO_2max depends to a large extent on the cardiovascular system. One of the major ways to determine cardiovascular fitness is to measure VO_2max.

VO_2max = (Stroke volume x Heart rate)max x a-vO_2 difference max

The stroke volume/heart rate product is referred to as cardiac output. This is the total amount of blood that the heart can pump per minute. From your results you will notice that your maximal heart rate has changed little, if at all. It is to be expected that maximal heart rate should change little if at all as a result of exercise training. One of the major effects of training is an increase in stroke volume. This increase in stroke volume is measureable up to a heart rate of ± 120 beats/minute, followed by a plateau. Further increases in cardiac output are accomplished via increases in heart rate. We did not determine stroke volume changes in your tests, but because your VO_2max increased, we can confidently predict that your stroke volume increased. The a-vO_2 difference refers to the ability of the body to extract oxygen from the arterial blood. This value usually improves by ± 5% as a result of training.

The following table lists the results of your two maximal exercise tests. These results are from the Day#1 of testing i.e., the test where we increased the work rate every minute until exhaustion, and where we continually sampled blood from an indwelling venous catheter to monitor changes in plasma lactate concentration.

Table 1. Training effects on measures of cardiovascular performance.

Variable	Your result Test #1	Test #2	Group mean Test #2
VO_2max (l/min)	2.92	3.47*	3.38
ml/kg/min	29.2	34.7*	39.93
VO_2max predicted	43.5	43.5	42.7
Heart rate max (bpm)	185	180	189
Work rate max (Watts)	240	280*	320

* denotes an improvement in your performance between test#1 and test#2.

The VO_2max predicted for you is that of a sedentary male of your age. It is recommended for purposes of cardiovascular fitness that you attain a value ± 10% above that predicted for you. This goal can be accomplished through a program of regular endurance type activity, similar to that which you participated in.

BODY COMPOSITION.

Body composition can be divided into two components: 1) lean body mass or fat-free weight (skeleton, water, muscle, connective tissue, organ tissues, teeth), and 2) body fat. The ideal body composition for men irrespective of age is to maintain a body fat of 16% or less. High levels of body fat are associated with an increased risk of coronary heart disease, stroke, hypertension, hyperlipidemia, diabetes, osteoarthritis, gallstones, gallbladder disease, renal disease, hepatic cirrhosis, accident proneness, surgical complications, and back pain. The following table reflects your body composition as predicted from skinfold measurements.

Table 2. Body composition before and after training.

Variable	Your result Test #1	Test #2	Group mean Test #2
Weight (kg)	100.0	100.0	86.5
% fat	29.2	28.5*	22.9
Fat weight (kg)	29.2	28.5*	19.8
Lean weight (kg)	70.8	71.5*	66.7

* denotes change from test#1 to test#2

At the ideal body fat percentage of 16% your weight would, based on the results of your 2nd test, be 85.1 kg. A combination of good nutritional habits and regular endurance type exercise will help you achieve this goal.

MUSCLE PERFORMANCE.

These results are based on the muscle biopsies taken before and after exercise on the Day#2 of the exercise tests. The data presented to you is that which we believe will be of interest to you. The photograph which accompanies this data reflects the distribution of fiber types in your muscle. The darker cells are the Type 1 fibers (slow-contracting or slow-twitch), and the pale cells are the type 2 fibers (fast-contracting or fast-twitch). The distribution of fiber type in the average sedentary male is 50% type 1 and 50% type 2.

It has been demonstrated that fiber type is to a large extent genetically determined, and is only marginally modifiable via exercise training. The fibers are however able to make significant changes in the way in which they utilize the various fuels stored within them and supplied to them. Any changes in fiber type observed during this study is probably due to differences in depth of biopsy, site of biopsy, and number of fibers counted. The following table shows your fiber type distribution, and the fuel utilization before and after training.

Table 3. Muscle fiber types and glycogen usage before and after training.

Variable	You		Group	
	test#1	test#2	test#1	test#2
Glycogen (mM/kg wet weight)				
Pre-ride	93	123	111	158
Post-ride	61	118	74	131
% change	**34**	**4**	**34**	**17**
Type 1 fiber (%)	37	50	45	46
Type 2 fiber (%)	63	50	55	54

Note that in the above table the ability to store muscle glycogen (glucose) is improved with training, and also that less glycogen is used during exercise when you compare trained to untrained individuals. This reflects a greater reliance on fat as a means of supplying energy to the contracting muscles. Note also that the group fiber type data has not changed significantly from the untrained to the trained state.

The major thrust of this project was to examine the effects of training on lactate kinetics using radioisotopes. The results we have obtained from processing of the blood samples seems to indicate that the major effect of training is a reduction in lactate production at low work rates, and an improved removal of lactate from the blood at higher work rates. The lower lactate concentrations seen in your blood were due to a combination of these effects after training. The mechanisms involved in these changes are yet to be determined.

Thank-you once again for being such a willing and compliant participant in this research project. I hope that I will one day be able to in some way reciprocate for your participation in this project. I hope that you will be able to put the results of your training to good effect, and that you will choose a lifestyle that incorporates regular endurance type exercise into it.

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