# HUMAN LACTATE KINETICS: TRAINING EFFECTS

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

by

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*This thesis is dedicated*

*to the memory of my mother*

*for all of the love and*

*encouragement she gave*

*during my childhood.*

*She managed to convince me*

*that I could do anything if*

*I set my mind to it,*

*and for that I will always*

*be grateful.*

# CONTENTS

# ABSTRACT

Blood lactate concentrations are lower at a given exercise intensity following training. The lower blood lactate concentrations have generally been attributed to a reduced rate of lactate production by the active skeletal muscles, but there is a growing body of evidence which suggests that the lower blood lactate concentrations may be due to an increased rate of lactate removal. The work described in this thesis used a variety of experimental manipulations to examine the effects of endurance exercise training on whole body blood lactate removal, and the production and removal of blood lactate by skeletal muscle.

A method for comparing rates of blood lactate removal between endurance-trained and untrained subjects during recovery from exercise was validated and used to compare lactate removal in endurance-trained and untrained cyclists during recovery from supine cycling exercise. It was found that the endurance-trained subjects not only exhibited lower blood lactate concentrations at a given work load during exercise, but they also demonstrated an increased rate of lactate removal during recovery compared with the untrained subjects. These findings were interpreted as indicating that the lower blood lactate concentrations in the trained subjects during exercise were, at least partly, due to an increased rate of blood lactate removal.

It had been suggested that trained skeletal muscle is the most likely site of increased lactate removal in endurance-trained subjects. To examine whether trained skeletal muscle does possess an increased ability to remove

lactate from the blood in comparison to untrained skeletal muscle, lactate removal by endurance-trained and untrained forearm skeletal muscle was compared during incremental venous sodium lactate infusion. No difference in lactate removal was found between trained and untrained skeletal muscle, suggesting that the increased lactate removal in trained subjects is not due to localised adaptations in the trained skeletal muscles.

Inactive skeletal muscle is a recognised site of blood lactate removal during exercise, and the possibility that systemic adaptations to training might increase lactate removal by inactive skeletal muscle during exercise, through the provision of a more favourable internal milieu, was investigated. Lactate removal by inactive untrained forearms was compared in endurance-trained and untrained cyclists during incremental supine cycling exercise and recovery. Rather than increasing lactate removal, systemic adaptations to training reduced the removal of lactate from the blood by inactive skeletal muscle. This was a result of adaptations in other tissues which served to lower blood lactate concentrations, thereby reducing the uptake and metabolic disposal of lactate by inactive skeletal muscle.

A decreased rate of net lactate output by trained compared with untrained forearms had been observed during handgrip exercise, and the question of whether the lesser net lactate output could be due to a decreased rate of lactate production, resulting from an increased utilisation of plasma FFA, was addressed. Trained and untrained forearms performed incremental handgrip exercise, and the trained forearms demonstrated a significantly lower net lactate

output at a given work load in comparison to the untrained forearms. The lesser net lactate output by the trained forearms occurred despite there being no differences in net plasma glucose or plasma FFA uptake between the two groups, and was attributed to a reduced glycogenolytic rate, probably resulting from an increased utilisation of FFA derived from some source other than the plasma.

In summary, this thesis has provided evidence that endurance training does not increase the ability of skeletal muscle to remove lactate from the blood, and that the lower blood lactate concentrations during exercise in the trained state result from the combination of a reduced rate of lactate production by the trained skeletal muscles and an increased rate of lactate removal by some tissue other than skeletal muscle.

# DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Jonathan David Buckley

Date: 23/5/97.

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# CHAPTER 1 - LITERATURE REVIEW

## 1.1 BACKGROUND

It has been recognised for many years that exercise can elevate the concentration of lactate in the blood (84). The extent to which the blood lactate concentration increases during exercise is a function of the intensity of the exercise and, amongst other things, the state of training, with concentrations being lower at a given absolute or relative exercise intensity following endurance training (34, 48, 90, 93, 98). The blood lactate concentration at any given time represents a balance between the rate at which lactate is produced and added to the blood and the rate at which it is removed. The degree to which the reduced blood lactate concentrations following training are due to either a decreased rate of lactate production or increased rate of removal is a source of continuing investigation. This review examines the literature relating to the production and removal of lactic acid during both exercise and recovery and how adaptations to training affect these two processes.

### 1.1.2 SOURCES OF BLOOD LACTATE ADDITION AND REMOVAL

Numerous tissues are involved in the regulation of blood lactate concentrations. Lactate can be produced and released into the blood by the skin (105), the intestine (186), the liver (40), the retina (171), the brain (171) and

skeletal muscle (40, 111), and removed from the blood and utilised by the heart (63), the liver (1), the kidney (217) and skeletal muscle (2, 190). Skeletal muscle and the liver therefore play dual roles in that they can release lactate into (40, 111) and remove lactate from (2, 190) the blood, with skeletal muscle believed to be the major tissue responsible for both of these processes during exercise (190).

## 1.1.3 PATTERNS OF BLOOD LACTATE ACCUMULATION

### 1.1.3.1 Incremental exercise

Despite it being well recognised that blood lactate concentrations increase during incremental exercise, there is considerable disagreement as to the precise pattern of this increase. The accepted dogma has been that, during incremental exercise, blood lactate concentrations increase slowly at first, before exhibiting an abrupt transition beyond which there is a rapidly accelerating increase in relation to work load (16). The exercise intensity at which this transition occurs has been termed the 'lactate threshold' (16, 103, 147, 160, 166, 206, 207). The existence of a lactate threshold has gained widespread acceptance, principally as a result of work carried out by Beaver et al., (16), who found that plotting the logarithm (log) of the blood lactate concentration against the log of oxygen uptake ($\dot{V}O_2$) during incremental exercise revealed a point at which there was a rapid transition (ie. a threshold) in the rate of increase of blood lactate relative to $\dot{V}O_2$. However, despite the

general acceptance of the concept of a lactate threshold, there is compelling evidence which suggests that, rather than exhibiting threshold like behaviour, blood lactate increases steadily as an exponential function of the work load during incremental exercise (96, 215). Hughson et al., (96) mathematically modelled the changes in arterial blood lactate concentration during incremental exercise using both the log-log method of Beaver et al., (16) and a continuous exponential function, and found that the pattern of blood lactate increase was better described by the continuous exponential function than the log-log model. Hughson et al., (96) also showed that the exponential function gave a random distribution of residuals about the line of best fit, whereas the log-log model gave a non-random pattern, indicating that use of the log-log model was inappropriate. These findings cast doubt on the validity of the concept of a lactate threshold, suggesting instead that blood lactate may increase progressively during incremental exercise as an exponential function of the work load.

#### 1.1.3.2 Continuous exercise

During continuous exercise at a given submaximal work load the extent to which the concentration of lactate in the blood increases is in large part dependent on the intensity of the exercise. At relatively low exercise intensities blood lactate can remain unchanged from resting values (39, 120). During moderate intensity exercise blood lactate concentrations increase

above resting levels due to an imbalance between the rates of lactate production and removal before reaching a steady-state as exercise continues and the rates of lactate production and removal become balanced (39, 120). However, during high intensity exercise, when the rate of lactate production exceeds the maximal rate of lactate removal, blood lactate will not reach a steady-state and will gradually increase until exercise ceases (120).

## 1.1.4 SIGNIFICANCE OF BLOOD LACTATE ACCUMULATION

### 1.1.4.1 Decreased performance

The factors which regulate blood lactate concentrations during exercise are of considerable interest because of a close association between lactate accumulation and muscle fatigue (54, 87, 129, 197). Lactic acid has a pK of approximately 3.7 (66) and is therefore more than 99.5% dissociated at physiological pH. It has been proposed that the hydrogen ions ($H^+$) which dissociate from the lactic acid molecule contribute to muscle fatigue by altering the binding of calcium ions ($Ca^{2+}$) to muscle regulatory proteins (115), or by inhibiting the activity of phosphofructokinase (PFK) (173). However, PFK inhibition by $H^+$ as a mechanism in fatigue during exercise has been questioned. Despite early studies demonstrating inhibition of PFK by $H^+$ *in vitro* (199), more recent studies, using *in situ* and *in vivo* preparations, have shown that PFK activity is maintained during intense muscular contractions (97, 142, 187)

despite decreases in muscle pH (187). Spriet et al., (187) proposed that PFK activity is maintained when *in situ* and *in vivo* preparations are used due to the presence of reduced inhibitor (ATP) and increased substrate (fructose 6-phosphate) concentrations which counter the $H^+$ induced inhibition.

In addition to potential $H^+$ mediated effects of lactate accumulation on fatigue, it has been shown recently that the lactate ion itself might also contribute to muscle fatigue. Hogan et al., (87) infused lactate into the blood perfusing an electrically stimulated dog gastrocnemius preparation and found that muscle tension was significantly reduced during the infusion despite no change in muscle pH. This finding implicates the lactate ion itself in the fatigue process, but the authors (87) were not willing at the time to speculate upon a possible mechanism of action.

### 1.1.4.2 Increased performance

Although the accumulation of high levels of lactate in the blood during exercise may be detrimental in terms of facilitating the onset of fatigue, it has been suggested that the accumulation of some lactate may be essential for the performance of high intensity exercise. Wasserman et al., (202) argued that the accumulation of lactate in the blood, or more precisely the accumulation of $H^+$ ions, may be essential for the performance of high intensity exercise because of the role of $H^+$ in increasing capillary $PO_2$ via the Bohr effect. A rise in capillary $PO_2$ would increase oxygen availability for cellular respiration, with a

concomitant increase in ATP synthesis and work output. Therefore, although the accumulation of high levels of lactate is associated with fatigue, the accumulation of some lactate may actually facilitate exercise performance.

### 1.1.4.3 Redistribution of carbon stores

Accumulation of lactate in the blood might also play an important role in the redistribution of carbon stores throughout the body. Ahlborg et al., (5) reported that during recovery from prolonged arm exercise, inactive leg muscle becomes an important site of lactate release to the splanchnic bed, and suggested that the Cori cycle might not only be involved in the transfer of lactate from exercising muscle to the liver for gluconeogenesis (38, 85), but might also involve lactate release by inactive skeletal muscle during and after prolonged exercise. The concept of lactate being used as a metabolic intermediate for the redistribution of carbon stores throughout the body was recognised a decade ago by Brooks (23) and referred to as the 'lactate shuttle'. The concept of the lactate shuttle, as espoused by Brooks (23, 25), not only encompassed the shuttling of lactate between tissues, but also between cells within a tissue. Brooks (23) argued that lactate could be released by fibres within a skeletal muscle which possess a high glycolytic capacity, and taken up by adjacent fibres within the same muscle which had a high oxidative capacity, without ever appearing in the venous blood. In support of this theory, Brooks (23) cited evidence from an isotopic tracer study which showed that only half of the lactate formed in working muscle was released into the venous circulation (194).

## 1.2 SKELETAL MUSCLE LACTATE METABOLISM

### 1.2.1 LACTATE PRODUCTION

The production of lactate by skeletal muscle during exercise is intimately linked to the contractile process. Upon the initiation of muscular contractions, calcium released from the sarcoplasmic reticulum activates phosphorylase *b* kinase converting glycogen phosphorylase (GPhos) from its inactive *b* form to the active *a* form (150). At the same time, although the ADP produced from the hydrolysis of ATP to provide energy for contraction is buffered by the creatine-phosphorylcreatine system (20) the cytosolic concentration of Pi increases. Pi stimulates PFK activity (151) and an increase in its cytosolic concentration, combined with the calcium mediated increase in GPhos activity results in a rapid increase in the glycolytic rate. Although the glycolytic rate can increase rapidly upon the initiation of muscular contraction, the activation of oxidative phosphorylation is much slower, being dependent on a decrease in the mitochondrial ATP/ADP ratio (150). Changes in this ratio are regulated by the phosphorylcreatine shuttle (20) and occur relatively slowly due to the functioning of this shuttle being dependent on creatine, which takes 1-2 minutes to reach steady-state (189). The combination of an increased glycolytic rate and an inadequate rate of pyruvate utilisation due to insufficient activation of oxidative phosphorylation and electron transfer can lead to increases in the cytosolic concentrations of pyruvate and NADH. Increases in the cytosolic pyruvate and NADH concentrations could increase lactate production through the mass action reduction of pyruvate to lactate by lactate dehydrogenase (LDH). As exercise

continues and creatine levels reach steady-state the activity of the phosphorylcreatine shuttle would increase, leading to a decreased mitochondrial ATP/ADP ratio. Such a decrease in the mitochondrial ATP/ADP ratio would in turn stimulate oxidative phosphorylation and electron transfer, allowing for an increased rate of transfer of reducing equivalents into the mitochondria and reducing cytosolic NADH concentrations. A reversal of calcium activation of GPhos, which has been shown to occur approximately 5 minutes after the onset of muscle contractions (36), would also assist in decreasing cytosolic NADH concentrations. The combined effect of these changes would be a gradual slowing of lactic acid production, which could lead to the attainment of a steady rate of lactate production and steady-state blood lactate concentrations. That is of course, provided that the rate of NADH formation in the cytosol had not exceeded its maximal rate of removal. If the work load was continually increased, or if the work load was so high that the rates of pyruvate and NADH formation exceeded their maximal rates of removal from the cytosol, lactate production and blood lactate concentrations would continue to increase.

#### 1.2.1.1 The role of hypoxia

The original demonstration of lactate production by skeletal muscle used inactive amphibian skeletal muscle in an anoxic environment (55), which led to the belief that skeletal muscle only produces lactate when hypoxic. The concept of hypoxia as the cause of lactate production by inactive muscle was later extended to lactate production by active skeletal muscle when Hill and Lupton

(84) demonstrated that lactate was produced by active skeletal muscle under hypoxic conditions. The notion of hypoxia as the cause of lactate production during exercise necessarily implies that when the oxygen supply in the mitochondria is not limiting, the ATP required by the working muscle is produced aerobically, but that oxygen availability in the mitochondria becomes insufficient to meet the ATP requirements of the working muscle as the work rate increases, with ATP production then being supplemented by an increase in the glycolytic rate. One of the assumptions inherent in such an argument is that the glycolytic rate increases in order to supplement aerobic ATP production. However, evidence presented by Sahlin et al. (175) suggests that lactate production is a poor means of supplementing aerobic energy production, accounting for only 2% of the total ATP turnover during steady-state submaximal bicycle exercise at approximately 70% of $\dot{V}O_{2\ max}$. Nevertheless, teleologically, a 2% greater energy yield resulting from lactate production could be significant in terms of species survival.

Whilst it is true that lactate production is enhanced during muscular exercise under hypoxic conditions, there is considerable evidence disputing hypoxia as the cause of lactate production during exercise. Blood lactate levels are lower at the same absolute submaximal work load following endurance training (93, 98) despite no change in $\dot{V}O_2$. If lactate production during exercise was caused by hypoxia the lower lactate levels seen in the trained state should be accompanied by a corresponding increase in $\dot{V}O_2$, yet this is not the case (83, 91, 216). Furthermore, blood lactate levels increase and decrease during respiratory hypoxia and hyperoxia respectively despite an unchanged $\dot{V}O_2$ (130).

That blood lactate concentrations can vary independently of $\dot{V}O_2$ tends to refute the suggestion that hypoxia is responsible for lactate production during exercise.

In addition to blood lactate being able to vary independently of $\dot{V}O_2$, it has been shown that lactate production occurs at a $PO_2$ in contracting muscle which is higher than that which impairs respiration (101), further suggesting that hypoxia is not the cause of lactate production during exercise. Idstrom et al., (101) concluded that respiration in isolated mitochondria is independent of oxygen availability when the $PO_2$ exceeds approximately 0.1 mmHg. Gayeski et al., (62) on the other hand, reported a slightly higher value of 0.5 mmHg for the $PO_2$ below which mitochondrial respiration is impaired, but they were reporting on *in situ* muscle preparations rather than isolated mitochondria, and work done by Kennedy and Jones (117) has indicated that intact cells require higher oxygen concentrations than isolated mitochondria to obtain the same state of reduction of cytochrome $a_3$. Despite both Idstrom et al., (101), and Gayeski et al., (62), both finding that mitochondrial respiration is not impaired at a $PO_2$ above 0.5 mmHg, Connett et al. (37) showed that lactate production occurred in dog gracilis muscle during submaximal work when $PO_2$ only decreased to about 2 mmHg. Similarly, Bylund-Fellenius et al., (29) found that $PO_2$ only decreased to about 7 mmHg in lactate producing gastrocnemius muscle. These findings indicate that lactate production occurs in actively contracting skeletal muscle at $PO_2$ values above those which have been shown to impair mitochondrial respiration, and suggest that it is unlikely that lactate production during submaximal exercise is caused by skeletal muscle hypoxia. However, it must be borne in mind that $PO_2$ could reach lower values in certain intracellular loci

than those reported by Connett et al., (37) and Bylund-Fellenius et al. (29) due to oxygen gradients between the capillaries and the mitochondria and a lack of homogeneity with regard to mitochondrial distribution within skeletal muscle (106).

### 1.2.1.2 Metabolic regulation of lactate production

Despite evidence that muscle $PO_2$ does not decrease below values which have been shown to impair mitochondrial respiration during exercise, Wilson et al., (211) suggested that a low $PO_2$ may have important metabolic consequences, even at values above those required to lower the respiratory rate. Koretsky and Balaban (123) demonstrated that apart from being dependent on $PO_2$, the mitochondrial respiratory rate is also dependent on the cytosolic phosphorylation potential [ATP/(ADP + Pi)] and the mitochondrial redox state [NAD/NADH]. The cytosolic phosphorylation potential regulates the respiratory rate as it is at near equilibrium with the first two sites of oxidative phosphorylation (50). However, because oxidative phosphorylation and electron transport are coupled reactions, changes in the respiratory rate are also regulated by substrate availability for the respiratory chain (ie. NADH). During high rates of respiration when aerobic metabolism is dependent on a relatively high $PO_2$, respiration is maintained constant by increases in NADH, ADP and Pi (211). These increases stimulate oxidative phosphorylation and electron transfer (50), resulting in an increased utilisation of molecular oxygen as the final electron acceptor for the electron transfer chain. An increased utilisation of

oxygen within the mitochondria establishes a greater diffusion gradient between the mitochondria and the extramitochondrial space (117), thereby maintaining mitochondrial oxygen uptake and respiration, even when extracellular $PO_2$ is falling (211). However, apart from maintaining mitochondrial respiration, increases in ADP, Pi and NADH also affect cellular metabolism. Elevated cytosolic ADP and Pi concentrations stimulate PFK (151, 208), and an increased mitochondrial NADH concentration decreases the capacity of the malate-aspartate shuttle to transport reducing equivalents into the mitochondria (116, 175). The combined effect of these two changes is an increase in cytosolic pyruvate and NADH concentrations, which would favour an increase in lactate production.

## 1.2.2 LACTATE REMOVAL

There is evidence, from isotopic tracer studies, that the rate of lactate removal is a saturable process (49, 193). Stanley et al., (193) reported a hyperbolic relationship between the rate of lactate removal and the blood lactate concentration during incremental exercise. Similarly, Eldridge et al., (49) found that the blood lactate concentration in anesthetised dogs increased more rapidly during lactate infusion than could be attributed to the tracer estimated rate of lactate entry to the vascular compartment and concluded that this was due to saturation of lactate removal. Studies using non-tracer methodologies have also reached similar conclusions with respect to lactate removal. Donovan and Pagliassotti (44) found that, during infusion of unlabelled lactate, the relationship

between the infusion rate and the arterial blood lactate concentration was best described by a second order regression. If the rate of addition of lactate to the blood was the sole determinant of the blood lactate response to exercise a linear relationship would have been expected between the rate of lactate infusion and the blood lactate concentration, and the finding of a curvilinear blood lactate response was interpreted as reflecting saturation of lactate removal at higher infusion rates. Ahlborg et al., (4) also interpreted an approximately exponential rise in arterial lactate concentration during continuous lactate infusion as indicating saturation of lactate removal.

#### 1.2.2.1 Lactate removal by skeletal muscle

Actively contracting skeletal muscle is not only a site of lactate production during exercise, but it can also be a site of lactate removal (82, 102, 107, 194). Inactive skeletal muscle has also been shown to remove significant quantities of lactate from the blood during both exercise (1, 3, 28, 31, 71, 124, 128, 163, 194) and lactate infusion (4, 32), with the rate of uptake being proportional to the arterial lactate concentration (28, 71). Although net lactate uptake by skeletal muscle implies net lactate disposal, Catcheside and Scroop (31) reported that all of the lactate taken up by inactive forearm skeletal muscle during moderate intensity leg exercise could be accounted for by passive uptake alone, with no significant metabolic disposal. Despite the finding of Catcheside and Scroop (31), numerous other studies (3, 4, 28, 32, 163) have found significant metabolic disposal of lactate taken up by inactive skeletal muscle.

The major pathways for the metabolic disposal of lactate removed from the blood by skeletal muscle include oxidation to $CO_2$ and $H_2O$ (26, 102), synthesis of muscle glycogen (8, 18, 27, 141, 183), and transamination to alanine and incorporation into proteins (27, 183).

### 1.2.2.1a Oxidation

Oxidation is a major avenue of lactate disposal during both rest and exercise, with the rate of lactate oxidation being linearly related to the metabolic rate (23, 138). Mazzeo et al., (138), monitored isotopic enrichment of $CO_2$ as a measure of lactate oxidation at rest and during both easy (51.6% $\dot{V}O_{2max}$) and hard (75.1% $\dot{V}O_{2max}$) exercise after the administration of a bolus of [1-$^{13}$C]lactate. These authors found that between 49.3% and 81.5% of lactate disposal could be accounted for by oxidation and concluded that oxidation is the major pathway for lactate disposal during both rest and exercise, with skeletal muscle, in particular slow twitch muscle, being suggested as the principal tissue responsible for the oxidation of the lactate. Jorfeldt (107), using active human skeletal muscle, and Pagliassotti and Donovan (156) using inactive rabbit skeletal muscle, also demonstrated that skeletal muscle is capable of oxidising lactate, and McDermott and Bonen (139) have suggested that oxidation is quantitatively the most important fate for lactate in both active and inactive muscle.

Studies which have examined the effect of recovery exercise on blood lactate have also provided evidence, albeit indirectly, of lactate oxidation by skeletal muscle. Studies using recovery exercise have demonstrated that the rate of blood lactate decline increases with the intensity of recovery exercise up to a critical level (17, 42, 149), and that the absolute $\dot{V}O_2$ of the exercise is significantly correlated (r=0.92) with the rate of blood lactate decline (140). The more rapid decline in blood lactate concentration during exercising recovery is presumably due to the active skeletal muscles oxidising the lactate as fuel for the exercise.

**1.2.2.1b Glyconeogenesis**

Early studies (141) suggested that significant glyconeogenesis from lactate could only occur in skeletal muscle after some degree of glycogen depletion. However, more recent studies have shown a role for glyconeogenesis in lactate removal by inactive non glycogen-depleted skeletal muscle (156, 183). Both Shiota et al., (183) and Pagliassotti and Donovan (156) demonstrated the incorporation of $^{14}C$ from lactate into glycogen under conditions of low net glycogen synthesis in isolated perfused skeletal muscle, indicating that net glycogen synthesis from lactate can occur in skeletal muscle in the absence of glycogen depletion. Furthermore, it has been shown that glyconeogenesis from lactate is optimal at pH 6.5-7.0 (22) and occurs at greater rates in fast-glycolytic muscle (156). This suggests a possible interaction

between fibre type and muscle [$H^+$] in determining the metabolic fate of lactate taken up by skeletal muscle.

### 1.2.2.1c Hypoxia

It has been suggested that the rate at which lactate can be removed from the blood by skeletal muscle may be influenced by the rate of lactate utilisation within the muscle (64). A rapid utilisation of lactate within a muscle would lower the intracellular lactate concentration, thereby establishing a greater blood to muscle lactate gradient and increasing the rate of lactate uptake from the blood.

Since lactate production is influenced by oxygen availability, with hypoxia increasing (86, 205) and hyperoxia decreasing (205) production, it would seem reasonable to assume that lactate utilisation might also be influenced by oxygen availability. However, despite there being no studies to date which have directly addressed the effect of oxygen availability on lactate utilisation, indirect experimental evidence does not support a role for oxygen availability in lactate removal. Stamford et al. (191) showed that there was no difference in the rate of blood lactate decline during exercising recovery between normoxic and hyperoxic conditions, and this also appears to be the case for hypoxic conditions (181), suggesting that the rate of lactate removal is independent of oxygen availability.

### 1.2.2.1d Substrate availability

Substrate availability has also been shown to exert a decisive influence on the rate of lactate utilisation by skeletal muscle. Essen et al., (51) demonstrated that lactate uptake was increased in skeletal muscle which had been glycogen depleted. The increased uptake was attributed to a reduced availability of muscle glycogen decreasing the glycolytic rate and reducing endogenous lactate production, thereby facilitating an increased blood-muscle lactate gradient which would promote the uptake of exogenous lactate.

In a different approach to examining the effects of substrate availability on lactate removal, Dunn and Critz (47) infused anaesthetised dogs with sodium lactate and either nicotinic acid to decrease arterial FFA levels, or a heparin and fat emulsion to increase arterial FFA, whilst measuring lactate uptake across the dogs' hindlimbs. They found a net uptake of lactate from the blood when arterial FFA concentrations were low, but that no lactate uptake occurred when arterial FFA were elevated. These findings were interpreted as indicating that the availability of an alternative substrate, namely FFA, can exert a significant influence on the rate of lactate uptake and utilisation by skeletal muscle.

### 1.2.2.1e Hormonal effects

Circulating hormones, in particular adrenaline, could significantly influence the rate of lactate utilisation by skeletal muscle. Adrenaline is a potent

activator of GPhos (164) and PFK (133), both of which are rate limiting enzymes in the glycolytic pathway (150). Activation of these two enzymes by adrenaline would increase the glycolytic rate, leading to an increased endogenous lactate production and a reduced blood-muscle lactate gradient. This would have the effect of reducing lactate uptake and possibly, lactate disposal. Such a theory is supported by the observation that lactate uptake and disposal by inactive skeletal muscle is enhanced by the infusion of propranolol, a general β-blocker (168).

### 1.2.2.1f Blood flow and capillarisation

The rate of lactate output by active skeletal muscle is influenced by muscle blood flow (70), but the role of muscle perfusion in lactate removal by inactive skeletal muscle is less clear. Both Ahlborg et al., (3) and Poortmans et al., (163) found that the rate of lactate uptake by inactive skeletal muscle was positively related to the arterial lactate concentration. However, Jorfeldt (107) found that the rate of lactate uptake by inactive skeletal muscle was more closely correlated with the product of arterial lactate concentration and the muscle blood flow ($r=0.94$) than with the arterial lactate concentration alone ($r=0.88$), which suggests that blood flow may be an important factor in determining the rate of lactate uptake by inactive skeletal muscle. Intuitively it would seem that an enhanced blood flow to inactive skeletal muscle would promote lactate uptake either by increasing the transit rate through the capillary bed, or increasing the number of perfused capillaries. An increased transit rate

would establish a higher extracellular to intracellular lactate gradient and a greater number of perfused capillaries would increase the surface area available for lactate uptake, both of which should lead to an enhancement of lactate uptake. However, current evidence does not support a role for blood flow in lactate removal by inactive skeletal muscle, it having been shown that lactate uptake by inactive skeletal muscle can vary independently of blood flow (66).

Given that the limiting surface area for lactate uptake is at the capillary, a greater capillary surface area should result in an increased capacity for lactate uptake. Capillary density is related to the fibre type distribution of a skeletal muscle, with slow twitch (red) muscle fibres being surrounded by a greater number of capillaries than fast twitch (white) muscle fibres (167). The capillaries supplying red fibres are also wider; about 5 microns as compared with 2.5 - 3.5 microns for those supplying white fibres (126). Thus, the total capillary surface area is considerably larger in the areas immediately surrounding red muscle fibres. Red fibres also have a greater capacity for oxidative metabolism than white fibres (9) which, combined with their greater capillary surface area, should result in a greater ability to take up and utilise lactate compared with white muscle fibres. Indirect support for this assumption was provided by Baldwin et al., (10), who showed that lactate oxidation is greater in red muscles (muscles composed predominantly of red muscle fibres) than in white muscles (muscles composed predominantly of white muscle fibres). Bonen et al., (21) also found evidence of an increased removal of lactate by red muscle fibres, reporting a low, but significant, correlation (r=0.54) between the rate of blood lactate decline

during recovery from exercise and the percentage of red muscle fibres in the previously active muscles.

## 1.2.3 LACTATE TRANSPORT IN SKELETAL MUSCLE

Until recently it was assumed that lactate moved across muscle membranes by simple diffusion (134), but there is now evidence that a membrane transport system for lactate exists (65, 110, 162, 172, 203). Large gradients between extracellular fluid and muscle during periods of elevated extramuscular lactate concentration (66, 203), and saturation kinetics for lactate uptake (107) were initially cited as evidence for the existence of a saturable lactate transporter in skeletal muscle membranes. However, such evidence was far from conclusive since it was acknowledged that blood to muscle lactate gradients could reflect a steady-state distribution of lactate determined by the transmembrane $[H^+]$ gradient, and saturation kinetics could reflect saturation of lactate utilisation rather than the saturation of a membrane transport system (64). Nevertheless, there is now strong evidence that a carrier-mediated membrane transport system for lactate exists in skeletal muscle. Juel (109) studied lactate output by mouse soleus muscles *in vitro* during recovery from electrically stimulated contractions and found that output was inhibited by the lactate transporter blockers p-chloromercuriphenyl sulphonic acid (PCMBS) and alpha-cyano-4-hydroxycinnamic acid (cinnamate), and that the lactate transporter was responsible for more than half of the lactate output. Mason and Thomas (136) studied the effect of cinnamate on lactate uptake by frog sartorius

muscle *in vitro* and found that cinnamate (2-5 mmol) caused a 39.1% inhibition in the peak rate of lactate uptake and, like Juel (109), suggested that the lactate transporter accounts for the majority of lactate movement across the sarcolemma. In an earlier study, which examined lactate uptake by mouse diaphragms *in vitro*, Koch et al., (121) also suggested that a lactate transporter accounts for the majority of lactate uptake, however, Koch et al., (121) went on to demonstrate that the transporter had a Km of 7.55 mM and saturated at about 10 mM.

Recently, lactate transport through the sarcolemma has been investigated using isolated sarcolemmal vesicle preparations (110, 161, 162, 169-172), and it has been found that lactate transport is mediated by a pH sensitive (170), saturable (171), sarcolemmal-bound carrier that co-transports lactate and hydrogen ions (110, 171) and has a Km of 40 mM (169). Whilst most previous studies had acknowledged that lactate transporters are responsible for the majority of lactate translocation across the skeletal muscle sarcolemma (109, 121, 136), Juel (110) indicated that the contribution of the lactate transporter to translocation across the sarcolemma is a concentration dependent process. Juel (110) estimated that the carrier-mediated component of lactate translocation accounted for ~90% of the total lactate flux across the sarcolemma at low lactate concentrations, which decreased to ~70% of the total flux at higher concentrations. This indicates that the diffusive component of lactate uptake might become increasingly important as a means of lactate translocation as the carrier-mediated component saturates at higher lactate concentrations.

Roth and Brooks (170) found that the transport rate for lactate into sarcolemmal vesicles was significantly diminished in the presence of an outwardly directed pH gradient, but observed similar rates of transport when internal and external pH were the same (ie no gradient) but varied in acidity, suggesting that the pH gradient across the sarcolemma, rather than the actual pH itself, is the critical factor determining the rate of transport. Roth (169) stressed that because the activity of the lactate transporter is dependent on the prevailing pH gradient, a decreased intracellular muscle pH would increase the rate of lactate and proton efflux from active skeletal muscle fibres, thereby helping to maintain intramuscular pH during exercise and delaying the onset of fatigue. Roth (169) also indicated that the efflux of protons from the active muscles would lower blood pH, which would then facilitate transporter mediated uptake of lactate by other tissues such as the heart liver, kidneys and inactive skeletal muscle. Based on these findings, it would appear that the pH dependent nature of lactate transporter activity would not only help to regulate intracellular pH, but would also assist in a more efficient distribution of lactate between the tissues as predicted by the lactate shuttle hypothesis (23, 24).

## 1.3. ENDURANCE TRAINING EFFECTS ON BLOOD LACTATE KINETICS

It has been shown repeatedly that the blood lactate concentration at a given relative or absolute work load is decreased following endurance training (34, 48, 90, 93, 98). However, the extent to which this decrease can be

attributed to either a reduced rate of lactate production or an increased rate of removal is the subject of some controversy.

### 1.3.1 DECREASED LACTATE PRODUCTION

Prior to the mid 1980s (81, 93), and even since (53), it has been generally accepted that the lower blood lactate concentrations during exercise in the trained state reflect a reduced rate of lactate production by the active muscles. Favier et al., (53) studied the gastrocnemius-plantaris-soleus muscle group in endurance trained and sedentary rats and found that after 3 minutes of electrical stimulation the increases in muscle lactate concentration and the decreases in muscle glycogen were not only smaller in the muscles of the trained rats, but the reduction in muscle lactate accumulation was proportional to the degree of glycogen sparing. These results were interpreted as providing direct evidence that decreased lactate production was responsible for the attenuated blood lactate response to exercise following training. Several other studies also showed that endurance training reduced carbohydrate utilisation (14, 165, 177) and muscle lactate accumulation (81, 112, 176, 177) during exercise, with the results being interpreted as providing evidence of a reduced rate of lactate production by endurance trained skeletal muscle.

### 1.3.1.1 Mitochondrial adaptations

There is considerable experimental evidence detailing biochemical adaptations to endurance training, which support the proposition that training lowers blood lactate concentrations by decreasing lactate production. Endurance training induces an increase in the mitochondrial content of skeletal muscle (88) as a result of gains in both the size and number of mitochondria (146). Underlying these increases in mitochondrial content are increases in the levels of the enzymes of the ß-oxidation pathway (145), the Krebs cycle (94) and components of the respiratory chain (88, 94), with an associated increase in the muscle's ability to oxidise pyruvate (146) and FFA (145).

The increases in mitochondrial content and respiratory enzyme levels resulting from training could contribute to a reduced rate of lactate production during submaximal exercise by various mechanisms, one of which is through tighter control of cytosolic adenine nucleotide concentrations. Holloszy (90) suggested that because the $\dot{V}O_2$ for a given absolute submaximal work load is the same in the trained and untrained states, and because mitochondria increase not only in size, but also in number following endurance training, the rate of electron transport and oxygen consumption per mitochondrion must be less in order to achieve a given $\dot{V}O_2$ in the trained state. It follows therefore, that because the oxygen consumption per mitochondrion must be less in the trained state the respiratory stimulus per mitochondrion, in terms of changes in the cytosolic phosphorylation potential, must also be less. Given that the glycolytic rate is regulated to a certain extent through allosteric regulation of

PFK by the cytosolic phosphorylation potential (137, 150, 151), attenuation of changes in this potential during exercise should lead to a reduced glycolytic rate and less lactate production. This reasoning is supported by the finding that, after training, muscle ATP decreases less at the same submaximal work load (111), and that a lower cytosolic ADP concentration is required to achieve any given rate of oxidative phosphorylation (46).

Endurance training also induces increases in the relative amount of mitochondrial-bound creatine kinase (MB-CK) in skeletal muscle (6). MB-CK forms an integral part of the phosphorylcreatine shuttle (20) and an increase in its activity would facilitate the translocation of ADP from the myofibrillar ATP-ase site into the mitochondria and the transfer of ATP in the opposite direction. An increased rate of translocation of these nucleotides would assist in minimising cytosolic ADP concentrations whilst maintaining high cytosolic ATP levels. These changes in cytosolic adenine nucleotides would further decrease stimulation of PFK, thereby reducing glycolytic flux and lactate production.

#### 1.3.1.2 Glycolytic enzymes

The formation of lactate from pyruvate and NADH is catalysed by the enzyme lactate dehydrogenase (LDH). LDH is a tetrameric molecule and two principal isoenzymes exist; LDH-M and LDH-H (41). LDH-H is found predominantly in the heart, has a lower Km for lactate than pyruvate, and therefore favours the conversion of lactate to pyruvate (41, 184). The M form of

LDH is found predominantly in skeletal muscle, has a lower Km for pyruvate than lactate and therefore favours the conversion of pyruvate to lactate (41, 184). Endurance training has been shown to decrease total LDH activity in skeletal muscle, with a shift in isoenzyme pattern toward LDH-H (185), which should lead to a reduced rate of lactate formation for any given rate of glycolytic flux.

The only significant endurance training adaptation in glycolytic enzymes apart from the change in LDH, is an increase in hexokinase activity (11). The significance of an increase in hexokinase activity in relation to a decreased rate of lactate production following training is unclear. However, an increase in hexokinase activity could lead to a greater proportion of carbohydrate being derived from blood glucose during exercise, which would contribute to the glycogen sparing effect which occurs in response to endurance training (93).

#### 1.3.1.3 The malate-aspartate shuttle

Endurance training might also attenuate lactate production by skeletal muscle through increases in the enzymes of the malate-aspartate shuttle. The inner mitochondrial membrane is impermeable to NAD and NADH (150) and the translocation of these pyridine nucleotides is mediated by the transfer of reducing equivalents via shuttle systems, of which the malate-aspartate shuttle is considered to be, quantitatively, the most important in skeletal muscle (151). Endurance training leads to increases in the enzymes of the malate-aspartate

shuttle (179, 180), which would increase the rate at which NADH can be removed from the cytosol for any given rate of NADH formation. Since NADH is a substrate for the LDH reaction, an increase in its rate of removal from the cytosol should reduce lactate formation.

#### 1.3.1.4 Alanine transaminase

Alanine transaminase catalyses the transamination of pyruvate to alanine (137), and its activity is increased through training (143). An increase in alanine transaminase activity would provide increased competition with LDH for pyruvate, with a consequent reduction in lactate formation.

#### 1.3.1.5 Fat metabolism

Arguably the most important adaptation to endurance training in terms of reducing lactate production during exercise is an increase in the capacity to oxidise fats for energy (99, 119, 122, 135, 144, 200). An increased capacity for fat oxidation enables a greater proportion of the energy required during submaximal exercise to be derived from fats with a proportional reduction in carbohydrate utilisation (158) through the operation of the glucose-fatty acid cycle (150). The greater mitochondrial content of trained muscle would provide a large surface area for the uptake of FFA which, combined with an increased concentration of ß-oxidation enzymes (145), would lead to an increased

production of acetyl-CoA from FFA. An increased production of acetyl-CoA from FFA, coupled with a training induced increase in citrate synthase activity (94) would raise the intra- and extra- mitochondrial citrate concentrations, which would in turn inhibit PFK activity (150). Inhibition of PFK would result in an increased concentration of glucose-6-phosphate in the cytosol which in turn would inhibit hexokinase (150). At the same time, the increased production of acetyl units from FFA would increase the acetyl-CoA/CoA ratio, leading to further inhibition of PFK, as well as inhibition of pyruvate dehydrogenase (PDH) activity (150). Since PFK, hexokinase and PDH are all rate limiting enzymes in the glycolytic pathway (150) their inhibition in such a manner would lead to a reduction in glycolytic activity, with a corresponding reduction in carbohydrate utilisation and possibly lactate production.

Despite it being well recognised that endurance training increases the capacity of skeletal muscle to utilise fats for energy during exercise, there is conflicting evidence regarding the source from which the additional FFA are drawn. Some studies have indicated that the elevation in lipid oxidation is a function of the trained skeletal muscles taking up more FFA from the plasma (119). However, other studies have reported either no difference (104), or even a decrease (135), in plasma FFA utilisation following training, and have instead attributed the greater oxidation of FFA to an increased utilisation of intramuscular triacylglycerols (99).

Due to the existence of a close relationship between the plasma FFA concentration and the rate of FFA oxidation (76, 157) an important factor

regulating FFA oxidation during exercise is the extent to which the plasma FFA concentration increases. However, despite an increased utilisation of FFA during exercise in the trained state, the plasma FFA concentration is lower at a given exercise intensity (99, 122, 135, 213); most likely due to a decreased rate of adipose tissue lipolysis since the levels of sympathoadrenal activity (79, 213), and the plasma concentrations of lipolytic hormones such as glucagon and growth hormone (79, 213), are markedly reduced by training. Given that the rate of FFA oxidation is related to the plasma FFA concentration (76, 157) it seems likely that, rather than increasing plasma FFA utilisation, training would decrease plasma FFA utilisation secondary to the decrease in plasma FFA concentration. However, Kiens et al. (119) found that trained skeletal muscle can extract more plasma FFA than untrained skeletal muscle at a given arterial concentration. Evidence of an increased capacity for trained skeletal muscle to extract FFA from the plasma was also provided by a study which compared plasma FFA uptake in trained and untrained legs during cycling exercise (81). Henriksson (81) had subjects with one trained leg and one untrained leg perform one-legged cycling exercise at work loads of 150-225 W and found that, despite working harder with the trained leg, the respiratory quotient was lower and plasma FFA uptake was higher when the trained leg was used for the exercise.

In direct opposition to the findings of Kiens et al., (119) and Henriksson (81), Hurley et al (99) reported no effect of training on the utilisation of plasma FFA. Hurley et al., (99) suggested instead that the greater fat oxidation during exercise in the trained state was the result of an increased rate of oxidation of intramuscular triacylglycerols. Hurley et al. (99) did not however, directly

measure plasma FFA utilisation, but instead estimated total FFA oxidation from RER measurements, and the contribution of intramuscular triacylglycerols to this total FFA oxidation from direct measurements of intramuscular triacylglycerol stores. Hurley et al. (99) found a lower RER and greater depletion of intramuscular triacylglycerol stores for a given exercise intensity and duration following endurance-training and concluded that the greater utilisation of FFA in the trained state was fuelled by increased lipolysis of intramuscular triacylglycerols. Further evidence suggesting that intramuscular triacylglycerols are the source of the greater fat oxidation during exercise is provided by the finding that training has been shown to increase the activity of type L hormone-sensitive lipase in rats (153), the enzyme believed to be responsible for triacylglycerol lipolysis in skeletal muscle.

#### 1.3.1.6 Blood flow

Quite apart from biochemical adaptations, endurance training also brings about adaptations in muscle blood flow which could contribute to decreased post-training blood lactate levels during exercise. Although blood flow to the exercising muscles, and therefore oxygen delivery, is unchanged, or in some cases even reduced during submaximal exercise following endurance training (7, 33, 178), there is a redistribution of this flow in trained muscle, with blood being directed away from fast-glycolytic (FG) fibres to regions composed of fast-oxidative glycolytic (FOG) fibres (7). This would have the effect of increasing the availability of FFA's to those fibres which have a greater capacity

to utilise them, further contributing to the increased fat utilisation following endurance training, with an associated decrease in carbohydrate utilisation and lactate production.

### 1.3.2 INCREASED LACTATE REMOVAL

#### 1.3.2.1 Isotopic tracers

In 1983 Donovan and Brooks (43), using an isotopic lactate tracer technique, reported that the lower blood lactate concentrations during exercise in the trained state were due to an increased rate of lactate clearance from the blood, with no change in the rate of lactate production. Since that initial study, numerous other studies (44, 45, 132, 159, 193, 195), also employing isotopic tracer techniques, have reported similar findings.

#### 1.3.2.2 Lactate infusion

The validity of using lactate tracer techniques for the study of lactate metabolism has been questioned on the basis that the labelled lactate rapidly equilibrates with endogenous pyruvate, such that calculations of lactate turnover might be more representative of pyruvate turnover (174, 214). This has given rise to the use of various non-tracer methodologies to examine the question of an increased capacity for lactate removal in the trained state. Donovan and

Pagliassotti (44, 45) infused lactic acid solutions into resting endurance-trained and untrained rats, and found that for a given rate of infusion the trained rats were able to maintain lower blood lactate concentrations. These were the first studies to demonstrate that a training induced enhancement of lactate removal was operative at rest. The greater lactate removal in the trained rats was attributed to increased oxidation and gluconeogenic cycling of lactate, with the trained skeletal muscles being proposed as the site of the increased lactate oxidation. However, given that a recent study carried out in this laboratory found no effect of training on the ability of inactive skeletal muscle to remove lactate from the blood (28), it seems unlikely that the trained skeletal muscles were the site of the increased lactate oxidation.

The increased lactate removal via gluconeogenesis reported by Donovan and Pagliassotti (44, 45) is indicative of a training effect on the liver or kidney. The importance of the liver in the elimination of lactate during exercise appears to be only minor, accounting for only 3-4% of total lactate removal during maximal intermittent exercise (69), and an increase in hepatic lactate removal with training appears unlikely given that *in vitro* studies have shown no changes in hepatic glucose production or the levels of hepatic gluconeogenic enzymes in response to training (212). This leaves the kidney as the most likely site of the increased gluconeogenesis reported by Donovan and Pagliassotti (44, 45), particularly since endurance training has been shown to increase gluconeogenesis from lactate in kidney cortex slices (125).

### 1.3.2.3 Rate of blood lactate decline during recovery

Studies in human subjects have also addressed the question of an increased rate of lactate removal following training by examining the rate of blood lactate decline during recovery from exercise. The assumption inherent in the use of this method is that an increased rate of blood lactate decline reflects an increased rate of lactate removal (15, 52, 57). Oyono-Enguelle (155) found correlational evidence of an increased rate of blood lactate decline relative to $\dot{V}O_{2max}$, but studies which have specifically compared rates of blood lactate decline between groups of trained and untrained subjects have been unable to demonstrate a more rapid rate of decline in trained subjects (15, 52, 57). Nonetheless, it has been argued (57) that differences in the rate of blood lactate decline during recovery have not been found in these studies due to the trained and untrained subjects working at different absolute work rates during exercise. It has been reported that the rate of blood lactate decline is inversely proportional to the absolute work load performed prior to recovery (59), and Freund et al., (57) suggested that if trained and untrained subjects ceased exercise at the same absolute work load a faster rate of blood lactate decline would be noted in trained subjects. However, in a reply to a letter to the editor published in the Journal of Applied Physiology (154), it was argued that the rate of blood lactate decline is not only inversely related to the work load performed prior to recovery, but is also inversely related to the level of blood lactate reached at the end of exercise, and it was suggested that the rate of blood lactate decline during recovery might be related to differences in blood lactate concentrations rather than work loads achieved at the end of exercise. This

point requires further investigation before any definitive conclusions regarding training effects on lactate removal can be drawn from studies examining the rate of blood lactate decline during recovery from exercise.

### 1.3.3 ALTERED LACTATE TRANSPORT

Early work, using rat skeletal muscle sarcolemmal vesicles, found no effect of training on the lactate transport capacity of skeletal muscle (172), but more recent work in both rats (162), and humans (161), has shown that training can increase sarcolemmal lactate transport by increasing the number and affinity of lactate transporter proteins. The difference in findings between the latter studies which found a training induced enhancement of lactate transport capacity (161, 162), and the earlier study which did not (172), could be related to differences in training intensity. Transporter adaptations are dependent on training intensity, with high training intensities being required for adaptations to occur (161, 162), and it is possible that the training protocol employed in the earlier study (172) was not sufficiently intense. It is difficult to compare the training protocols employed in the different studies since two used rats and the other used humans as subjects. The problem is further complicated by the fact that whilst the two studies which used rats both used treadmill running protocols, one used training sessions incorporating 120 min of continuous treadmill running (172), whilst the other used an interval training protocol (162). However, it is noteworthy that the rat study which demonstrated an increase in lactate transporter activity (162) used a maximum running speed of 3.6 $km \cdot hr^{-1}$, which

was more than twice the maximum of 1.6 $km \cdot hr^{-1}$ used in the study which showed no effect of training (172).

## 1.4 SUMMARY AND AIMS OF THE THESIS

The question of whether the lower blood lactate concentration at a given work load following endurance training is due to an increase in lactate removal or a decrease in lactate production is a complex one. The majority of evidence tends to favour decreased lactate production, but there is a growing body of evidence, from both isotopic tracer and non-tracer studies, which suggests a role for an increase in lactate removal.

The aim of this doctoral study was to investigate firstly whether endurance-trained subjects have an increased capacity for lactate removal, and secondly, to examine the role played by skeletal muscle in any such increased removal. Specifically, the aim was to test the dual hypotheses that (1) endurance-trained subjects possess an increased capacity for lactate removal, and (2) that skeletal muscle is the site of that increased removal.

# CHAPTER 2 - GENERAL METHODS

## 2.1 GENERAL

The subjects used in the studies described in this thesis volunteered to participate after being informed of all of the risks involved. All studies were approved by the Committee on the Ethics of Human Experimentation at the University of Adelaide and were conducted in an air-conditioned laboratory under thermoneutral conditions (21 ± 1°C) with subjects in the supine position. All subjects were studied in the morning after an overnight fast.

## 2.2 THE FOREARM AS A MODEL FOR THE STUDY OF SKELETAL MUSCLE METABOLISM

The forearm has been widely used as a model for the study of the metabolism of both inactive (4, 28, 31, 163), and active (77, 80, 108, 201) skeletal muscle. The forearm provides a model well suited for such studies since arterial and venous blood vessels can be readily catheterised, and blood samples from the deep forearm veins derive almost exclusively from forearm muscle both at rest (35, 148) and during handgrip exercise (100).

## 2.3 EXPERIMENTAL TECHNIQUES

### 2.3.1 Measurement of forearm circumferences and volumes

Forearm circumferences were measured at a point 10cm distal to the olecranon process using a 5 mm wide measuring tape (Dean, Fibreglass).

Forearm volumes were determined by immersing the forearm into a cylinder containing water at a temperature of 20 ± 1°C. The cylinder was designed such that as the limb was inserted the displaced water ran off through a spigot into a container resting on a set of computerised force transducer scales (Toledo Digital Scales, capacity 120 ± 0.02 kg). In order to determine forearm volumes, the hand was first immersed until the water was level with the middle wrist crease, and the volume of water displaced by the hand was then weighed. Once the water displaced by the hand had been weighed the forearm was immersed to a point 10 cm proximal to the olecranon process. The volume of water displaced was again weighed and the forearm volume was determined by subtracting the weight of the water displaced by the hand from the total weight of water displaced by the hand and forearm. Forearm volume was then calculated from the weight of the water displaced, assuming that the density of the water was 1.0.

### 2.3.2 Collection of blood samples

All catheters, both arterial and venous, were inserted under local anaesthesia (lignocaine hydrochloride 2%, Xylocaine) at least 20 min prior to the commencement of blood sampling.

In order to obtain samples of arterial blood, Cathlon IV 20G catheters were inserted into the brachial artery of one arm at the elbow, with the catheter tip directed upstream.

For collection of venous blood samples, Insyte 18G catheters were inserted into the deep muscle branch of the antecubital vein of the appropriate arm, with the catheter tip directed upstream.

All blood samples were taken anaerobically into glass syringes which were immediately capped and placed on ice until aliquotted appropriately. To maintain blood volume and patency of the catheters, each blood sample was immediately replaced with an equivalent volume of normal saline.

### 2.3.3 Measurement of heart rate

Heart rate (HR) was recorded continuously during all protocols as 15 second averages with a Sport Tester PE 3000 heart rate microcomputer and

chest transmitter (Polar Electro). Both HR and the electrocardiogram were also displayed on a Nihon Kohden Lifescope 6 intensive care unit video monitor.

### 2.3.4 Measurement of arterial blood pressure

Apart from the periods when arterial blood was being sampled, both pulsatile and mean arterial blood pressures were measured continuously throughout each protocol. This was achieved by attaching the extension tubing of the arterial catheter to a Gould Statham P23 ID strain-gauge pressure transducer connected to a Grass model 7B polygraph.

### 2.3.5 Measurement of forearm blood flow

Forearm blood flow (FBF) was measured in all of the studies reported in this thesis using venous occlusion plethysmography (74, 209). The human forearm is comprised mainly of three tissues, muscle, skin and bone, each of which has its own blood supply. Venous occlusion plethysmography (74, 209) records the sum of the blood flows through all three of these tissues, and it is not possible to determine the contribution of each tissue individually to the overall metabolism of the forearm. Despite this limitation, the conclusions derived from the present work may be interpreted as reflecting essentially skeletal muscle metabolism given the greater bulk and metabolic rate of this tissue in comparison to skin or bone.

Venous occlusion plethysmography (74, 209) is used to estimate blood flow through a limb segment from changes in limb volume when the venous drainage is occluded, with the assumption being that the rate of blood flow through the limb is proportional to the rate of change in limb volume. Percentage changes in the volume of the limb are deduced from measurements of the relative change in limb circumference by attaching mercury in silastic strain gauges at a particular point. For the experiments described in this thesis, Medasonics SG24 strain gauges were placed 10 cm distal to the olecranon process and venous occlusion cuffs were placed 10 cm proximal to the olecranon process of each arm in which FBF was to be measured. The inflation procedures for the occlusion cuffs varied according to whether the study involved handgrip exercise or not (see 2.3.5a and 2.3.5b). Electrical output from the strain gauges was amplified using a Medasonics SPG16 Strain-Gauge Plethysmograph and recorded as limb circumference changes by a Toshin two-channel pen recorder. The strain gauges were calibrated at the end of each experiment by progressively stretching them known increments of length.

### 2.3.5a Measurement of blood flow in the inactive forearm

During measurement of FBF in the inactive forearm the hand circulation was occluded by a pneumatic wrist cuff inflated to 220 mmHg (127, 210). The venous occlusion cuff was inflated to a pressure of 75 mmHg and deflated in 20 second cycles, being inflated for 13 seconds during flow measurements and

deflated for 7 seconds between measurements, such that 3 recordings were taken each minute. The elbow was flexed such that the upper arm formed an angle of ~160° with the forearm, which was elevated ~20° to horizontal to ensure adequate venous drainage between flow measurements. FBF values were calculated from the average of 3 inflow curves and expressed as $ml \cdot min^{-1} \cdot 100ml$ of $forearm^{-1}$.

### 2.3.5b Measurement of blood flow in the active forearm

Venous occlusion plethysmography was not only used to measure blood flow through the inactive forearm, it was also used to measure FBF during handgrip exercise. During handgrip exercise the arterial inflow curves were subject to movement artefacts as a result of contraction of the forearm muscles (see Fig. 2.1). To allow for estimation of the slope of the arterial inflow curves despite these movement artefacts, subjects were instructed to completely relax their forearms between contractions, particularly when performing high work loads. The slope of the arterial inflow curve was then determined from the slope of the points corresponding to the times when the forearm was relaxed between each contraction. More specifically, FBF was estimated from the slope of a line drawn along the bottom of the first 3 points of each curve, as indicated in Figure 2.1. Arterial inflow curves in which any of the 3 points used to determine the slope deviated from linearity were excluded since this indicated that the forearm had not been completely relaxed between contractions.

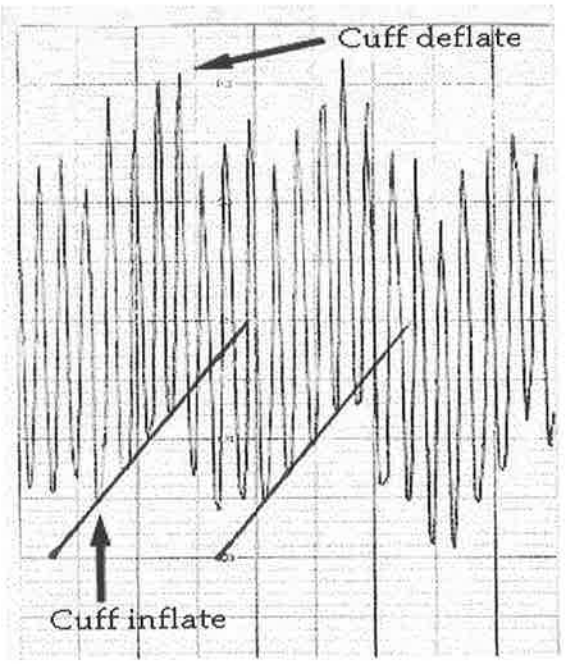


Fig. 2.1. Estimation of the slope of arterial inflow curves during handgrip exercise from lines drawn along the bottom of the first 3 points of each curve.

The circulation to the hand was not occluded by a pneumatic wrist cuff when measuring FBF during studies involving handgrip exercise in order to avoid ischemically-induced pain in the hand. During such studies, the hand circulation was also left unoccluded whilst the forearm was inactive during recovery so that the measurements of FBF during these periods related to the same tissue volume as when FBF was measured during handgrip exercise.

## 2.3.5b.i Validity of using venous occlusion plethysmography for the measurement of blood flow in contracting limb segments

Venous occlusion plethysmography was previously used by Barcroft and Dornhorst (12) to measure blood flow through the human calf during rhythmic exercise. However, they found that the use of this technique underestimated actual blood flow due to the pumping action of the muscles forcing venous blood back under the occlusion cuff. To overcome this problem they developed a method for quantifying the amount of blood forced under the cuff, but this method did not lend itself well to the recording of repeated measurements.

To determine the validity of using venous occlusion plethysmography for the measurement of FBF during handgrip exercise a preliminary study was carried out to determine whether venous blood would escape under the venous occlusion cuff during FBF measurements.

### Methods

Eighteen forearms, including those used in the experiments reported in chapters 4 and 5, were used in this preliminary study. A venous occlusion cuff was placed 10 cm proximal to the olecranon process of the arm in which FBF was to be measured. The cuff was inflated to a pressure of 75 mmHg for 8 seconds and then deflated for 7 seconds, allowing for four cycles per minute. An Insyte 18G catheter was inserted into the deep muscle branch (148) of the

antecubital vein of the arm being investigated to record changes in venous blood pressure (VBP) as the venous occlusion cuff inflated and deflated during handgrip exercise. This was achieved by attaching the extension tubing of the venous catheter to a Gould-Statham P23ID pressure transducer, which was in turn connected to a Grass model 7B polygraph. Once the catheter was inserted and the venous occlusion cuff was in place VBP was measured during an initial 2 minute rest period to obtain baseline data. VBP was then measured whilst subjects performed incremental handgrip exercise to exhaustion as described at 2.4.2.

**Results**

When the occlusion cuff was inflated, VBP increased in synchrony with systolic contractions of the heart when the forearm was at rest, and with the contractions of the forearm muscles during handgrip exercise (see Figure 2.2).

There were eight inflation periods during each work load, seven during exercise and one during the intermission between work loads when the resistance was adjusted on the dynamometer. The peak VBP values for each of the seven inflation periods during exercise at a given work load were similar and so the average of the seven values was taken to represent the peak VBP for that work load. Peak VBP increased as a result of handgrip exercise from a resting value of 12.6 ± 1.1 mmHg to a peak of 55.8 ± 1.2 mmHg at the end of

exercise (Figure 2.3), which was significantly below the venous collecting cuff pressure of 75 mmHg ($P<0.001$).

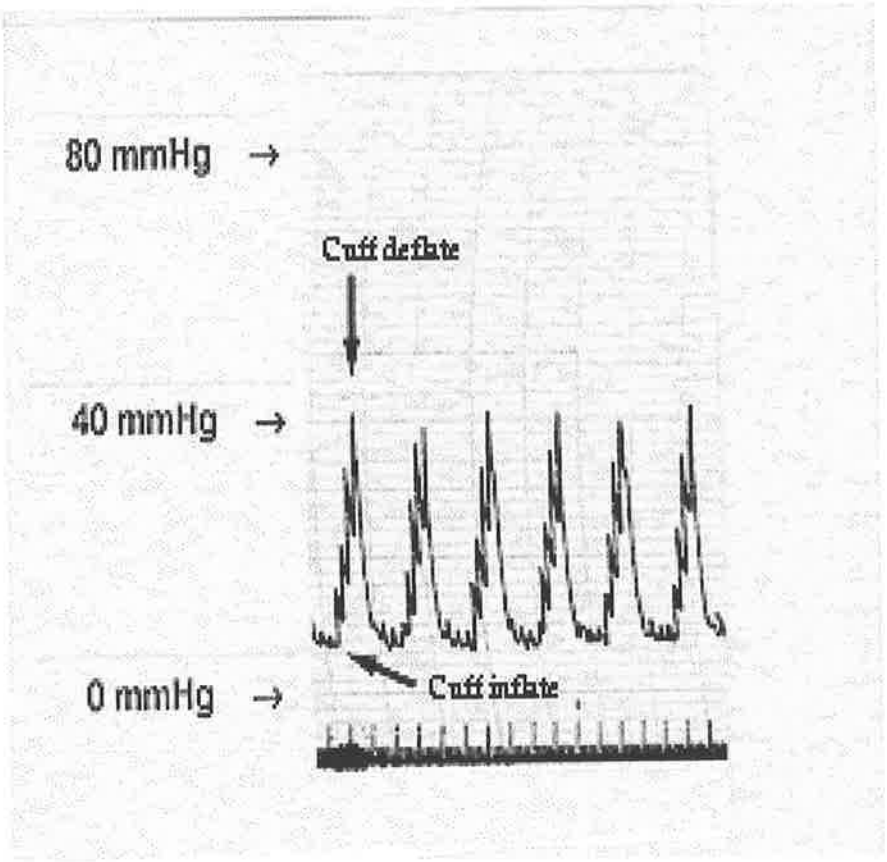


Fig. 2.2. Changes in blood pressure in the antecubital vein with inflation and deflation of a venous occlusion cuff during handgrip exercise.

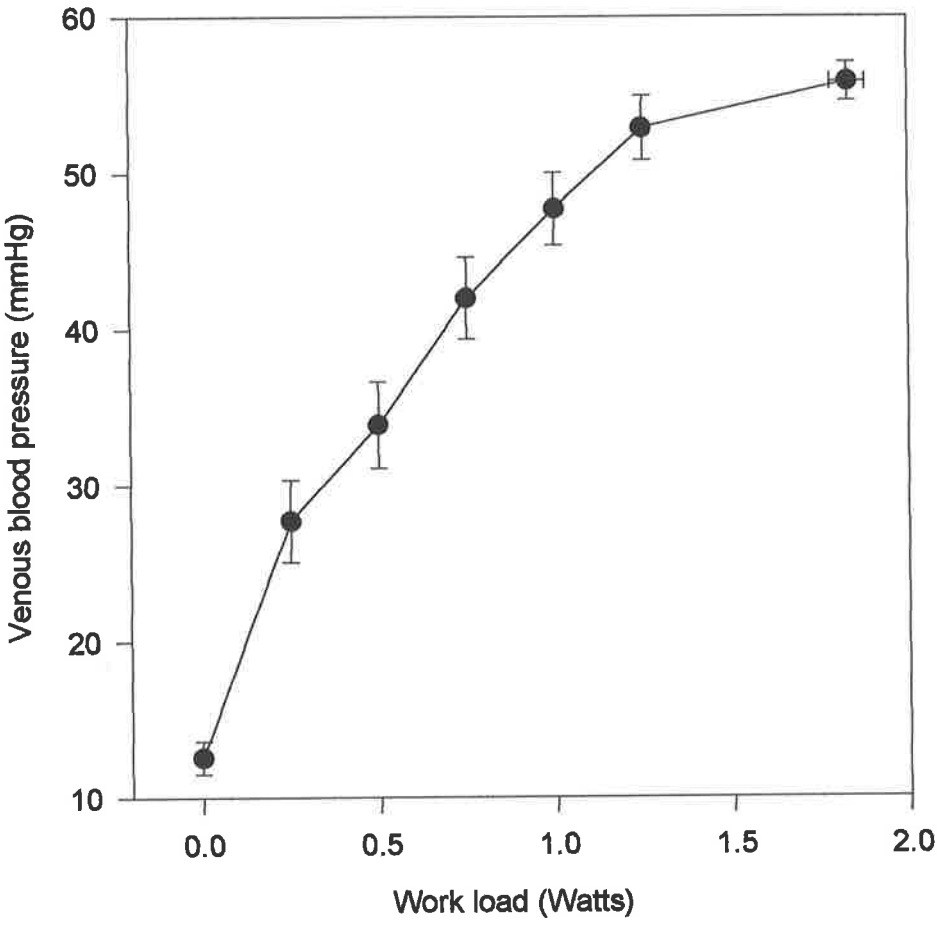


Fig. 2.3. Blood pressure in the antecubital vein during incremental handgrip exercise (n=18). Values are means ± SEM.

## Discussion

The use of venous occlusion plethysmography for the measurement of blood flow during rhythmic contraction of the muscles in a limb segment will underestimate actual flow if venous blood is forced under the occlusion cuff by the pressure developed as a result of the pumping action of the active muscles. However, venous blood will only be forced under the occlusion cuff if VBP exceeds the pressure in the cuff. Strandell and Shepherd (196) indicated that for measurements of FBF using venous occlusion plethysmography in resting forearms, venous collecting pressures between 35 to 80 mmHg give the same slopes of arterial inflow. In the present study a cuff pressure of 75 mmHg was used, and it was found that VBP remained significantly lower than this (55.83 ± 1.22 mmHg), even during maximal work loads ($P<0.001$). It should also be noted that these pressure readings represented the VBP recorded at the tip of the catheter, which was positioned approximately 15 cm upstream from the occlusion cuff. It is likely that VBP at the periphery of the occlusion cuff would have been less than the values recorded at the catheter tip due to additional resistance offered by the 15 cm of venous lumen and any valves which may have been interposed between the catheter tip and the occlusion cuff, thereby further reducing the likelihood that blood could be forced under the cuff during handgrip exercise.

Barcroft and Dornhorst (12) reported that blood was forced under the venous occlusion cuff when venous occlusion plethysmography was used to measure calf blood flow during rhythmical plantar flexion and dorsiflexion of the

ankle. The difference in the findings between the study by Barcroft and Dornhorst (12) and the present one could be related to differences in the size of the muscle groups used during exercise. The calf muscles are a relatively large muscle group in comparison to the muscles of the forearm. Contraction of larger muscle groups such as the calves would result in the forceful expulsion of a larger volume of blood from the muscle per contraction than during exercise with smaller muscle groups such as in the forearm. The expulsion of a larger volume of blood per contraction could lead to a more rapid increase in venous blood pressure in a limb segment isolated by a venous occlusion cuff. However, the actual VBP reached would also depend on the contraction rate, the length of time the collecting cuff was inflated and the capacitance of the vascular system within the limb segment. Nevertheless, based on the findings of the present study it would appear that a collection time of 8 seconds and a contraction rate of 30 contractions·min$^{-1}$ leads to the development of a peak VBP of only 56 mmHg during maximal handgrip exercise, indicating that a collecting cuff pressure of 75 mmHg is more than adequate for measurements of FBF when the forearm is either at rest, or during handgrip exercise. A collecting cuff pressure of 75 mmHg is not so high that it will affect the slope of the arterial inflow curve when the forearm is at rest (196), nor is it so low that venous blood will be forced underneath the collecting cuff during handgrip exercise. Accordingly, a collecting cuff pressure of 75 mmHg was used during all measurements of FBF for the studies described in this thesis.

## 2.4 EXERCISE TESTS

### 2.4.1 Determination of maximum handgrip strength

Maximum handgrip strength (MHS) was determined using an isotonic handgrip dynamometer (Smedley). MHS was always determined immediately after forearm circumferences and volumes had been measured and at least 45 min prior to the performance of handgrip exercise. The contraction path for the dynamometer was set at 30 mm. Subjects were instructed to stand erect with their arms to their sides and to then grip the dynamometer as hard as possible whilst maintaining their elbow in an extended position, without allowing either their forearm, or the dynamometer, to come into contact with any other part of their body. MHS was defined as the greatest force that could be achieved out of three attempts.

### 2.4.2 Incremental handgrip exercise

All incremental handgrip exercise tests were performed using a custom-made isotonic handgrip dynamometer. The dynamometer was spring-loaded and incorporated cams which were designed to ensure that a constant resistance was maintained throughout the range of contraction.

During handgrip exercise the dynamometer was positioned such that the upper arm formed an angle of approximately 160° with the forearm, which was

elevated approximately 20° to horizontal in order to facilitate venous drainage between measurements of FBF. To ensure that only the forearm was used during exercise a 45° wedge was placed between the trunk and the upper arm, effectively isolating the forearm muscles by preventing shoulder and upper arm movement. The contraction distance for the dynamometer was set at 30 mm and the order of forearm exercise was randomised.

A ten minute rest period always preceded handgrip exercise to allow for resting blood samples to be taken and basal measurements to be recorded. Subjects then performed rhythmic handgrip exercise at a rate of 30 contractions·min$^{-1}$, in time to a Nikko Seiki mechanical metronome. Exercise periods lasted 1 min 45 sec, with a 15 sec pause at the end of each period to allow for adjustment of the dynamometer resistance. Initial work loads were always set at 0.25 W with 0.25 W increments until exhaustion occurred. Exhaustion was defined as the time at which the subject ceased exercise of their own volition, or when the subject was unable to move the handgrip through the full range of motion on two successive contractions.

### 2.4.3 Measurement of peak systemic oxygen uptake

Peak systemic oxygen uptake ($\dot{V}O_{2peak}$) was determined using an incremental exercise test on a Jaquet supine cycle ergometer. Pedal rate was set at 60 rpm with an initial work load of 100 W. The work load was incremented by 25 W every three min until the subject reached exhaustion.

Expired air from the expiratory port of a 2700 series Rudolph valve was directed via 1 m of large bore tubing (Vacumed Clean-Bor) to a 2.6 litre mixing chamber (Sportech, A.C.T.). Dried gas was sampled continuously from the mixing chamber and passed to oxygen (Morgan Rapid Zr) and carbon dioxide (Beckman LB-2) analysers calibrated with gas mixtures authenticated by analyses with the micro-Scholander apparatus. Inspired volume was measured using a P.K. Morgan (Mark 2, large turbine) ventilometer and the electrical outputs from the ventilation meter and gas analysers were passed to an IBM-compatible personal computer, which calculated the necessary ventilatory variables at 30 sec intervals using standard algorithms and ASYST-based software developed in this laboratory.

## 2.5 BIOCHEMICAL ASSAYS

### 2.5.1 Blood lactate

Blood samples taken for determination of blood lactate concentrations were immediately deproteinised in ice-cold 8% (w/v) perchloric acid (PCA), using a 1:2 dilution (ie., 1 ml of blood into 2 ml PCA). Samples were then centrifuged for 10 min at 1500 *g* and the supernatant was drawn off and frozen at -20°C for subsequent analysis of lactate using an enzymatic ultraviolet endpoint method (Sigma Diagnostics 826-A). The coefficient of variation for the determination of blood lactate concentrations was 0.037.

### 2.5.2 Plasma free fatty acids

Blood samples taken for determination of plasma FFA concentrations were immediately placed into tubes containing 7.5 mg of dipotassium EDTA and kept on ice until the end of the experiment. Samples were then centrifuged for 10 min at 1500 *g* and the plasma was drawn off and frozen at -20°C for subsequent determination of plasma FFA concentrations using an enzymatic calorimetric method (Wako, 990-75401). The coefficient of variation for the determination of FFA concentrations was 0.02.

### 2.5.3 Plasma glucose

Blood samples taken for determination of plasma glucose concentrations were immediately placed into tubes containing 7.5 mg of dipotassium EDTA and kept on ice until the end of the experiment. Samples were then centrifuged for 10 min at 1500 *g* and the plasma was drawn off and frozen at -20°C for subsequent determination of plasma glucose concentrations using an enzymatic calorimetric method (Sigma Diagnostics 510-A). The coefficient of variation for the assay of plasma glucose concentrations was 0.009.

### 2.5.4 Blood gases

Blood samples taken for determination of blood gases were kept on ice in capped glass syringes until they were analysed for $PO_2$, $PCO_2$, pH, [*t*Hb] and $\%SO_2$ using a Radiometer Copenhagen ABL510 blood gas system. The longest a sample was kept on ice before being analysed was approximately one hour, and preliminary testing had indicated that, provided glass syringes were used, samples could be kept on ice for more than two hours with no change in blood gas concentrations.

## 2.6 SODIUM LACTATE INFUSATE SOLUTION

The 4 M sodium lactate infusate solution used for the experiments described in Chapter 5 was prepared by the pharmacy of the Queen Elizabeth Hospital, South Australia. The method of preparation was as follows:

207 g of sodium hydroxide A.R. was dissolved in 480 ml of water for injection, and then added to 424 ml of an 85% (w/v) lactic acid solution A.R. This solution was then boiled for one hour.

After the solution had cooled the pH was titrated to 7.0 ± 0.02 using a dilute hydrochloric acid solution B.P., which had itself been made up by adding 23 ml of concentrated hydrochloric acid (36% w/w) to 72.6 ml of water for injection.

The resultant sodium lactate solution was filtered through a 0.2 micron Acro 50A filter into 100 ml glass bottles (60 ml per bottle) and sealed with a Merco bung, a screw cap and a dust cap. The bottles of infusate solution were then sterilised by autoclaving at 121°C for 30 min and stored for 30 days before being tested for particulate matter using an Allen viewer or "Maggy" lamp. A sample of the solution was then assayed to confirm its concentration, and each 1000 ml of infusate solution was found to contain 4000 mM of sodium, and 4000 mM of bicarbonate (as lactate).

## 2.7 CALCULATIONS

### 2.7.1 Uptake/output of substances by the forearm

In calculating net forearm uptake and/or output of substances from differences between arterial and venous blood or plasma concentrations there is an inherent assumption that the venous concentrations are representative of the average concentrations of the total venous drainage of the forearm musculature. In reality this is not the case, and it should therefore be borne in mind that such quantitative calculations must be regarded as approximations only.

Values for the net uptake and output of lactate by the forearm were calculated from the product of FBF and the veno-arterial blood lactate concentration differences. Values for net uptake and/or output are generically

referred to as net lactate flux, with negative values indicating net uptake and positive values net output.

Values for the net forearm uptake and/or output of substances measured in the plasma (eg. glucose and FFA) were calculated using the following formula:

$$\text{Net uptake/output} = (C_v \times (FBF \times 1 - Hct_v)) - (C_a \times (FBF \times 1 - Hct_a))$$

where $C_v$ = the concentration of the substance in the venous plasma

FBF = forearm blood flow

$Hct_v$ = venous haematocrit (expressed as a decimal)

$C_a$ = the concentration of the substance in the arterial plasma

$Hct_a$ = arterial haematocrit (expressed as a decimal)

Use of the above equation took into account differences between arterial and venous haematocrit as water moved into or out of the blood as it traversed the forearm musculature. Again, values were generically referred to as net flux, with negative values indicating net uptake and positive values net output.

### 2.7.2 Net production and disposal of substances by the forearm

The total net production or disposal of a particular metabolite by the forearm was estimated from the area under a point-to-point curve of net flux

taken from the end of the initial rest period to the end of recovery. Provided that the arterial and venous metabolite concentrations, and FBF, had returned to resting values by the end of the recovery period, the total net production or disposal of a given metabolite by the forearm was assumed to be represented by the net area under this curve.

### 2.7.3 Carbon dioxide and oxygen content of blood

The carbon dioxide content [$CO_2$] and oxygen content [$O_2$] of blood samples were determined from [*t*Hb], %$SO_2$, $PO_2$, $PCO_2$ and pH using the Henderson-Hasselbalch equation (204) and the equations of Severinghaus (182) respectively.

### 2.7.4 Plasma volume changes

Percentage changes in plasma volume were calculated according to the method of Harrison (78), with the blood sample obtained at the end of the initial rest period prior to experimental manipulation being used as the point of reference.

## 2.8 STATISTICS

Normality of distribution of data was tested using the Kolmogorov-Smirnov test, with a Lilliefors significance level (188). Group differences in normally distributed data were analysed using the independent *t*-test, and group differences in non-normally distributed data were analysed using the nonparametric Mann-Whitney test.

Time series measurements between groups were compared using two-way analysis of variance (ANOVA) for repeated measures incorporating a Greenhouse-Geisser adjustment for multisample asphericity (131). Ludbrook (131) indicated that when analysing data using repeated measures ANOVA it is an assumption that the data possess a property known variously as multisample sphericity, circularity or compound symmetry. Multisample sphericity is a condition in which serially repeated measurements have the same variance at each repetition and the same degree of correlation with each other over all repetitions, or in other words, it is a requirement that the variance-covariance matrix is symmetrical. This requirement is rarely fulfilled and unless a correction is made there is an increased risk of making a type 1 error (ie. increased risk of making a false positive inference). A correction for a lack of multisample sphericity can be made by adjusting the degrees of freedom associated with F, using a measure of multiple sample sphericity called $\varepsilon$ (epsilon). Greenhouse and Geisser (75) determined a method by which $\varepsilon$ could be calculated from the sample variance-covariance matrix. There are a variety of computerised

statistical programs that will carry out the necessary calculations of $\varepsilon$, and SPSS for Windows (188) was used to calculate $\varepsilon$ in the present series of experiments.

Correlation and regression analyses were carried out using a computerised spreadsheet and statistical program (Microsoft® Excel 5.0).

For all statistical tests the level of significance used throughout was $P \leq 0.05$ unless otherwise stated.

# CHAPTER 3 - EFFECT OF TRAINING ON WHOLE BODY LACTATE REMOVAL

## 3.1 INTRODUCTION

Blood lactate concentrations are lower at a given absolute or relative submaximal exercise intensity in endurance-trained individuals. In general, these lower blood lactate concentrations have been attributed to decreased lactate production by the active skeletal muscles (90, 93, 178). However, there is some evidence that the lower blood lactate concentrations during exercise in the trained state may be due to an increase in the rate at which lactate is removed from the blood (43-45, 57, 132, 193, 195).

Studies using isotopic tracers in both animals and humans have reported an increased capacity for lactate removal in the trained state during exercise (43, 132, 193, 195), as well as under resting hyperlactatemic conditions (44, 45). However, the use of lactate tracers has been criticised on the basis that the majority of the lactate label is lost as a result of rapid equilibration between lactate and pyruvate, such that lactate turnover more accurately reflects pyruvate rather than lactate flux (174, 214).

Given the criticisms associated with the use of lactate tracers certain non-tracer methodologies have been used to examine the question of an increased capacity for lactate removal in the trained state. Infusions of

unlabelled lactate have been shown to elicit lower arterial blood lactate concentrations in trained compared with untrained rats (44, 45), with the lower blood lactates being attributed to enhanced lactate removal. Studies in human subjects have examined the rate of blood lactate decline during recovery and suggested that an increased rate of decline would reflect an increased capacity for lactate removal (15, 52, 57). There is some evidence of an increased rate of blood lactate decline in endurance-trained subjects (155), but studies which have specifically compared rates of blood lactate decline between groups of trained and untrained subjects have been unable to demonstrate a greater rate of decline in trained subjects (15, 52, 57).

The aim of the present study was to determine whether endurance-trained subjects exhibit an increased rate of blood lactate decline during recovery from exercise. Arterial blood lactate data during recovery were described using a biexponential time function (15, 56, 57, 60, 61, 218, 219) and the rate constants for the decline in blood lactate were compared for endurance-trained and untrained subjects.

## 3.2 METHODS

**3.2.1 *Subjects and experimental conditions:*** Six untrained male subjects (UT) and six competitive endurance-trained (TR) male cyclists volunteered to participate in the study. The descriptive characteristics of the subjects are reported in Table 3.1.

Prior to experimentation a Cathlon IV 20G catheter was inserted under local anaesthesia (lignocaine hydrochloride 2%, Xylocaine) into the brachial artery of the left arm at the elbow with the tip directed upstream.

Exercise and recovery were achieved in the supine position. A 10 min rest period preceded exercise. Incremental exercise was performed on a mechanically-braked Jaquet supine cycle ergometer. Pedal rate was set at 60 rpm with an initial work load of 100 W. The work load was increased by 25 W every 10 min until volitional exhaustion. Measurements of oxygen uptake were made every 30 sec during the initial 10 min rest period and throughout the whole of the exercise period.

**3.2.2 *Blood samples:*** Arterial blood samples (3.0 ml each) were collected at 5 and 10 min of the initial 10 min rest period, every 2.5 min during exercise and immediately on the cessation of exercise. Post-exercise blood samples were obtained at min 2.5, 5, 7.5 and 10 and every 10 min thereafter up to 120 min of recovery for analysis of lactate and blood pH. Due to technical difficulties blood pH data for one UT subject were not collected.

**3.2.3 *Blood lactate:*** Individual blood lactate recovery curves were fit by the following biexponential time function using an iterative nonlinear regression technique:

$$La(t) = A_1(1-e^{-\gamma_1 t}) + A_2(1-e^{-\gamma_2 t}) + La(0)$$

where La(*t*) ($mmol \cdot l^{-1}$) was the lactate concentration at any *time t*, La(0) ($mmol \cdot l^{-1}$) was the lactate concentration at the end of exercise, and $A_1$ and $A_2$ ($mmol \cdot l^{-1}$) were the amplitudes of the fitted exponential function (15, 56, 57, 59, 60, 155). It has been proposed that the rate constants $\gamma_1$ ($min^{-1}$) and $\gamma_2$ ($min^{-1}$) reflect the effectiveness of lactate redistribution from the previously worked muscles to the blood and the overall ability to remove lactate from the blood during recovery (15, 57, 59).

## 3.3 RESULTS

**3.3.1 *Exercise performance:*** The TR subjects demonstrated significantly higher values for peak oxygen uptake (Table 3.1) and performed more work than the UT subjects during the incremental exercise test (UT, 304.4 ± 41.6 kJ; TR, 638.1 ± 45.1 kJ; $P<0.01$), with the UT group reaching volitional exhaustion at 179.2 ± 10.0 W and the TR group at 241.7 ± 8.3 W ($P<0.01$).

**Table 3.1.** ***Anthropometric and physiological characteristics of subjects.***

| | UT Subjects (n=6) | TR Subjects (n=6) |
|---|---|---|
| Age (yr) | 25.0 ± 2.0 | 19.6 ± 0.4† |
| Height (cm) | 174.5 ± 2.1 | 172.8 ± 4.0 |
| Mass (kg) | 68.4 ± 3.3 | 68.7 ± 1.1 |
| Peak oxygen uptake | | |
| $l \cdot min^{-1}$ | 2.6 ± 0.2 | 3.7 ± 0.2* |
| $ml \cdot kg^{-1} \cdot min^{-1}$ | 38.8 ± 3.2 | 53.2 ± 2.3* |

Values are means ± SEM. * Significantly different from untrained ($P < 0.01$). † Significantly different from untrained ($P < 0.05$).

**3.3.2 *Blood Metabolites:*** The blood lactate concentrations during rest, exercise and the subsequent recovery period are shown in Fig. 3.1. Prior to exercise the blood lactate concentrations were not different between the two groups (UT 0.7 ± 0.1 $mmol \cdot l^{-1}$; TR 0.8 ± 0.1 $mmol \cdot l^{-1}$). As soon as exercise commenced the blood lactate concentration in the UT subjects began to rise above resting levels, whereas in the TR subjects this did not occur until 37.1 ± 5.3 min of exercise at a work load of 183.4 ± 12.4 W. At a given work load during submaximal exercise the blood lactate concentration was lower in the TR group ($P<0.01$), but the values achieved by the end of exercise were not different (UT, 7.7 ± 0.9 $mmol \cdot l^{-1}$; TR, 5.1 ± 1.7 $mmol \cdot l^{-1}$). Peak blood lactate concentrations (UT, 8.8 ± 1.2 $mmol.l^{-1}$; TR, 5.4 ± 1.7 $mmol.l^{-1}$) were reached at

1.3 ± 0.6 min and 2.5 ± 1.1 min of recovery in the UT and TR groups respectively, with no differences in either the peak values or time to peak.

The parameters for the curve fitting of blood lactate concentrations during recovery are given in Table 3.2. The biexponential time function fit the data significantly better than a single exponential function ($P<0.0001$).

**Table 3.2.** ***Parameters of biexponential curve fits to individual blood lactate recovery curves.***

| | UT Subjects (n=6) | TR Subjects (n=6) |
|---|---|---|
| $A_1$ ($mmol \cdot l^{-1}$) | 7.524 ± 4.047 | 3.223 ± 1.110 |
| $\gamma_1$ ($min^{-1}$) | 0.251 ± 0.063 | 1.533 ± 0.650 |
| $A_2$ ($mmol \cdot l^{-1}$) | -14.467 ± 4.550 | -7.622 ± 2.766 |
| $\gamma_2$ ($min^{-1}$) | 0.055 ± 0.005 | 0.092 ± 0.012* |
| La(0) ($mmol \cdot l^{-1}$) | 7.7 ± 0.9 | 5.1 ± 1.7 |

$A_1$ and $A_2$, amplitudes of fitted exponential function; $\gamma_1$ and $\gamma_2$ velocity constants of fitted exponential function; La(0), arterial lactate concentration at end of exercise (*time* 0 of recovery).

* Denotes significantly different from UT subjects ($P<0.05$).

The curve fit accounted for 97% of the observed variance for the UT group and 95% for the TR group with no difference in goodness-of-fit between the two groups. The $\gamma_2$ values were significantly higher for the TR group but neither $\gamma_1$ nor the amplitudes $A_1$ and $A_2$ were different. The values achieved for $\gamma_2$ appeared to be independent of both La(0) ($r = 0.45$, $P>0.05$) and the final absolute work rate ($r = 0.45$, $P>0.05$). The blood lactate concentration in both groups had returned to pre-exercise levels by the end of recovery.

Arterial blood pH during rest, exercise and the subsequent recovery period are shown in Fig. 3.2. Prior to exercise, blood pH was not different between the two groups (UT 7.411 ± 0.004; TR 7.416 ± 0.006). During exercise blood pH decreased in both groups and was significantly lower in the UT group at a given work load during submaximal exercise ($P<0.05$). At the end of exercise, blood pH [pH(0)] had decreased to 7.284 ± 0.024 in the UT group and 7.335 ± 0.029 in the TR group, these values not being different statistically. During recovery there were no differences in blood pH between the two groups, with the lowest values occurring at 3.0 ± 0.9 min (UT) and 3.8 ± 1.4 min (TR) of recovery, reaching values of 7.239 ± 0.027 and 7.316 ± 0.029 in the UT and TR groups respectively.

La(0) and pH(0) exhibited a significant negative relationship ($r = -0.82$, $P<0.01$) such that higher values for La(0) were associated with lower values for pH. pH(0) was also correlated with $\gamma_2$ in both groups of subjects ($r=0.74$, $P<0.01$) such that lower values for pH(0) were associated with lower values for $\gamma_2$ (Fig. 3.3). It was considered appropriate to pool the data for the

relationship between pH(0) and $\gamma_2$ since there was no statistical difference in this relationship between the two groups.

## 3.4 DISCUSSION

Although previous studies have indicated an increased rate of decline in blood lactate for trained subjects during active recovery from exercise (17, 52), and there is some correlational evidence suggesting that trained subjects have higher $\gamma_2$ values than untrained subjects during resting recovery (155), this is the first study to provide direct evidence of a more rapid decline in blood lactate concentration in endurance-trained subjects during resting recovery from exercise.

It has been proposed (59) that the ability to remove lactate is reflected in the rate constant $\gamma_2$, with a larger value, as found in the TR group in the present study, indicating an enhanced removal capacity. However, although it has been widely accepted that a greater rate of blood lactate decline during recovery from exercise reflects a more rapid rate of lactate removal (15, 52, 57, 59), the rate of blood lactate decline will not only be influenced by the rate at which lactate is removed from the blood, but also by the rate at which it is added to the blood by the previously active skeletal muscles. It is possible therefore, that a more rapid decline in blood lactate concentration during recovery from exercise may not necessarily be the result of a more rapid lactate removal, but could instead be due to a more rapid decrease in the rate at which lactate is added to the blood

by the previously active skeletal muscles. However, evidence presented in Chapter 4 of this thesis indicates that there is no difference in the kinetics of lactate output by previously active trained and untrained skeletal muscle during recovery from exercise, suggesting that a greater rate of blood lactate decline during recovery from exercise does reflect a more rapid rate of lactate removal.

Oyono-Enguelle et al. (155) found a positive relationship between maximal aerobic power and $\gamma_2$ and interpreted this as reflecting a greater ability to remove lactate in trained subjects. More recent studies (15, 57) have undertaken direct comparisons of the rates of blood lactate decline in trained and untrained subjects during recovery from exercise, but have been unable to demonstrate any effect of training on $\gamma_2$. Despite no evidence of an elevated $\gamma_2$, Freund and coworkers (57) argued that endurance-trained subjects do possess an increased capacity for lactate removal. These authors reasoned that because of an inverse relationship between $\gamma_2$ and absolute work rate (59, 155), trained subjects may not exhibit a greater $\gamma_2$ unless they cease exercise at the same absolute work load as untrained subjects. In addition to the negative relationship between $\gamma_2$ and absolute work rate reported by Freund et al., (59) and Oyono-Enguelle et al., (155), when the published data from these two studies were analysed further, additional negative relationships between La(0) and $\gamma_2$ were found [Freund et al. (59), $r = -0.78$, $P<0.001$; Oyono-Enguelle et al., (155), $r = -0.89$, $P<0.001$]. A similar analysis of published data from another study by Freund et al., (58) also revealed an inverse relationship between La(0) and $\gamma_2$ ($r = -0.76$, $P<0.001$). That analysis of the data from all three studies revealed similar negative relationships suggests that La(0) may play an

important role in determining the rate of blood lactate decline, a point which has been argued by Bassett et al., (15). All previous studies that have compared rates of blood lactate decline in trained and untrained subjects have reported significant differences between groups in either La(0) (52, 57) or peak lactate concentrations immediately post-exercise (15), with trained subjects generally exhibiting higher values. Due to the negative relationship between La(0) and $\gamma_2$ that is evident from the data of previous studies (58, 59, 155), if, as in the present study, these lactate concentrations had been similar in both trained and untrained subjects, higher $\gamma_2$ values might have been observed for the trained subjects.

An explanation as to how elevated blood lactate concentrations could lead to lower values for $\gamma_2$ is not immediately apparent, particularly since in the present study no relationship was found between La(0) and $\gamma_2$. First principles would suggest that an elevated lactate concentration should lead to higher rather than lower values for $\gamma_2$ since an elevated blood lactate concentration should increase the reaction velocities of the enzymes involved in lactate removal. However, the negative relationship that was found between blood lactate concentration and blood pH ($r = -0.82$, $P<0.01$) suggests a possible mechanism whereby the lower pH values associated with higher blood lactate concentrations could lead to increased allosteric inhibition of the enzymes involved in lactate removal, since many of these enzymes are inhibited by decreased pH (19). Considering that pH(0) was similar in both groups in the present study a proportional enzymatic inhibition would be expected in both subject groups, which would leave a greater enzymatic capacity in the trained

subjects since the tissue concentrations of many of the enzymes involved in lactate removal are increased with training (89, 92). Inhibition of similar numbers of enzymes in both subject groups would leave a greater proportion of the total enzyme pool uninhibited in trained subjects, facilitating an increased rate of lactate removal. The finding of a significant relationship between pH(0) and $\gamma_2$ in the present study provides experimental support for such a proposal, and since no relationship was found between La(0) and $\gamma_2$ it is possible that pH might play a more important role in determining the rate of blood lactate decline following exercise than does the arterial blood lactate concentration.

The increased rate of lactate removal during recovery in the TR group was associated with lower blood lactate concentrations at a given work load during submaximal exercise. Since the blood lactate concentration at any given time represents a balance between the rates of lactate appearance and disappearance a lower blood lactate concentration at a given work load could be the result of a decreased rate of lactate production and/or an increased rate of lactate removal. It has been suggested that $\gamma_2$ indirectly informs on the lactate removal ability prevailing during exercise (57). Bearing this in mind, the findings of the present study offer support for the proposal that increased lactate removal contributes to the lower blood lactate concentrations seen in trained subjects during exercise.

In conclusion, the present study demonstrates that the rate of blood lactate decline during recovery from incremental exercise to volitional exhaustion is more rapid in trained subjects, indicating an increased capacity

for lactate removal. The greater lactate removal is associated with lower blood lactate concentrations during exercise, and may also reflect a greater ability for trained subjects to remove lactate from the blood during exercise.

## 3.5 FIGURES

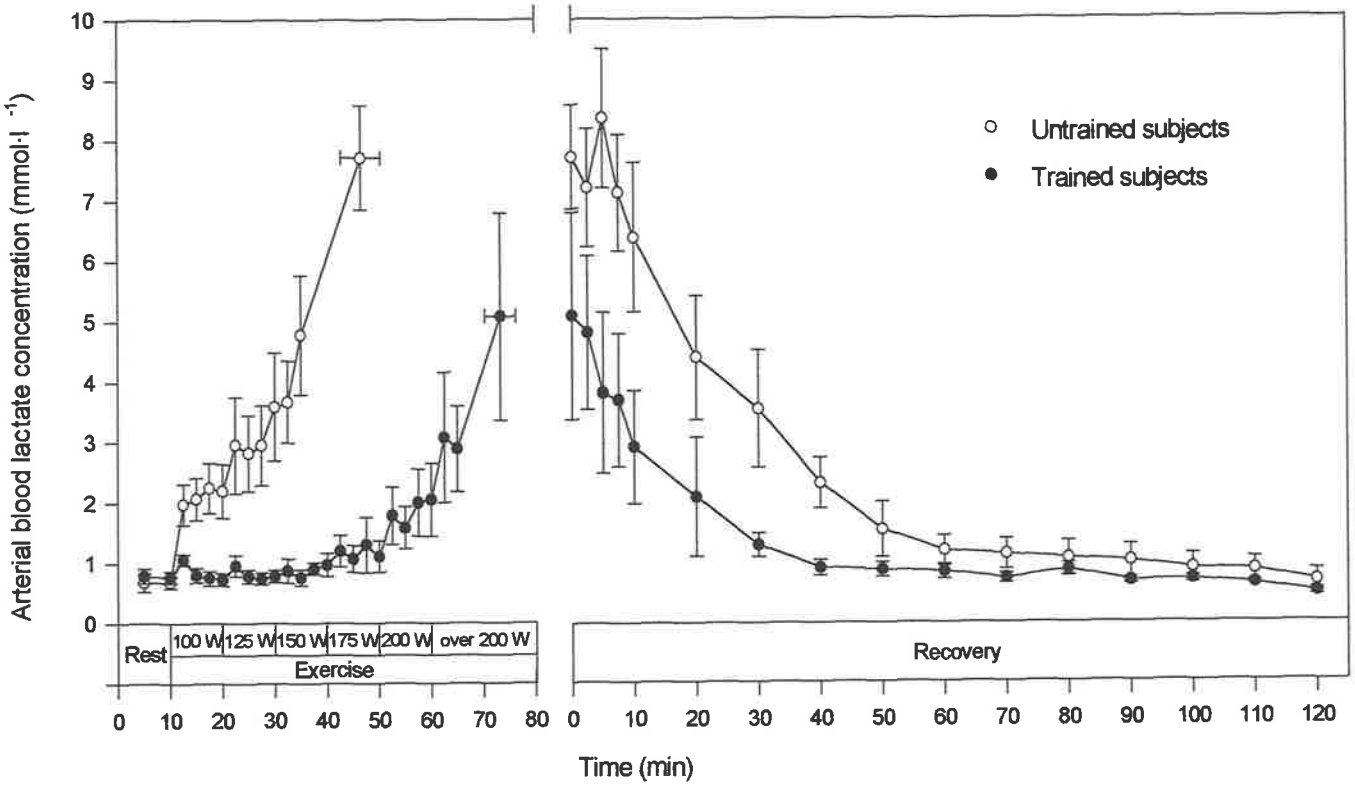


Fig. 3.1. Arterial blood lactate concentrations during rest, incremental supine cycling exercise and recovery in untrained (n=6) and endurance-trained (n=6) subjects. Values are means ± SEM.

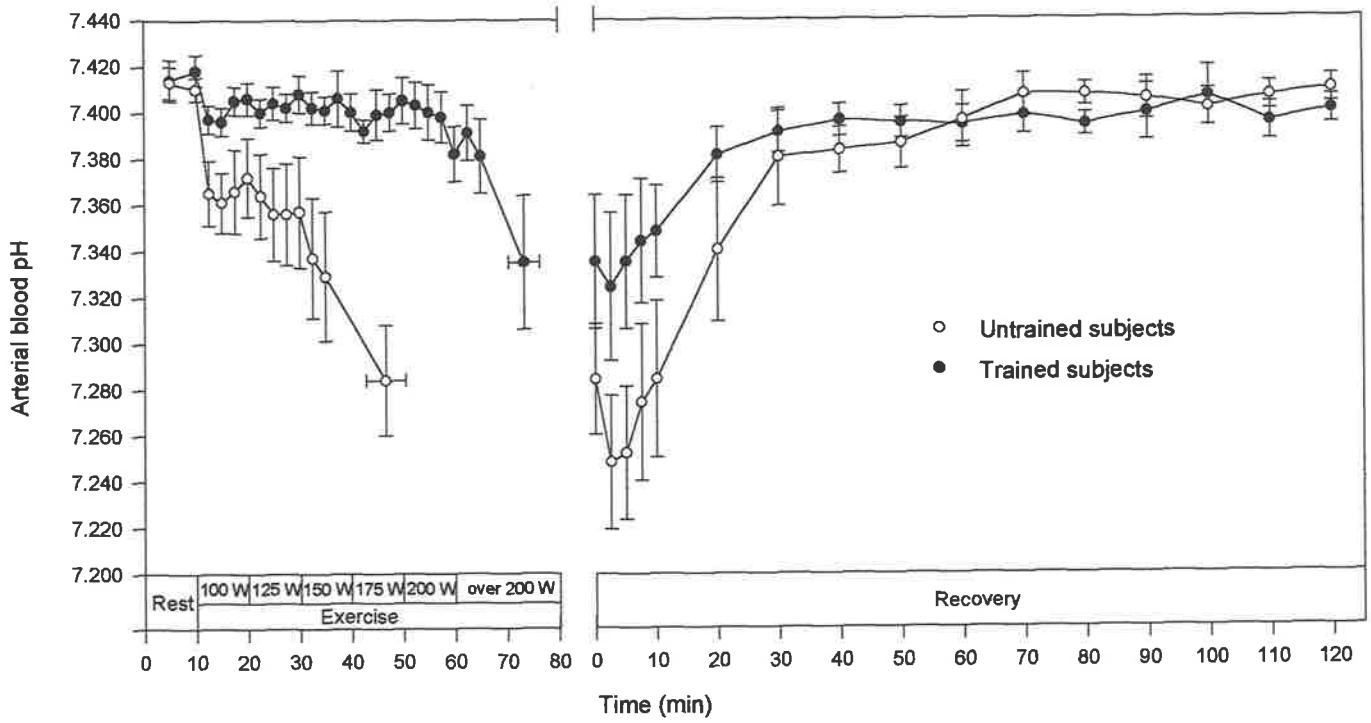


Fig. 3.2. Arterial blood pH during rest, incremental supine cycling exercise and recovery in untrained (n=5) and endurance-trained (n=6) subjects. Values are means ± SEM.

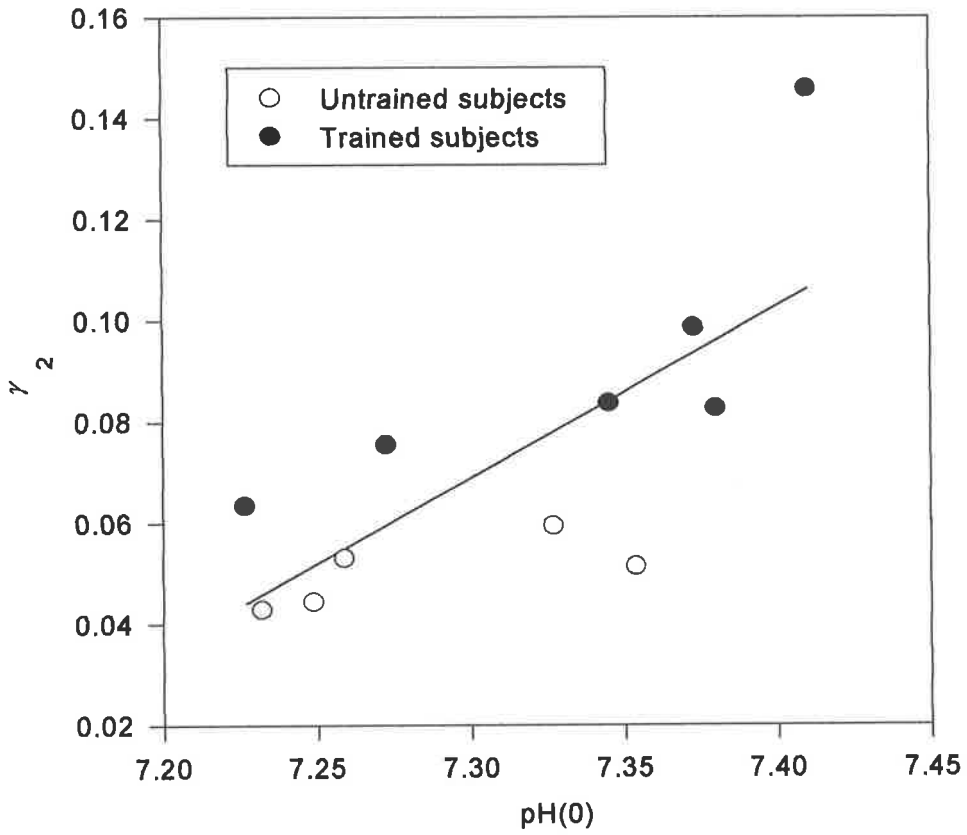


Fig. 3.3. The relationship between pH(0) and $\gamma_2$ in untrained (n=5) and endurance-trained (n=6) subjects. Values are means ± SEM.

# CHAPTER 4 - EFFECT OF TRAINING ON LACTATE OUTPUT BY SKELETAL MUSCLE

## 4.1 INTRODUCTION

Previous studies in human subjects, including the work described in Chapter 3 of this thesis, have examined the effect of endurance training on the rate of blood lactate decline during recovery from exercise and have suggested that an increased rate of blood lactate decline reflects an increased rate of lactate removal (15, 52, 57, 155). However, prior to the work described in Chapter 3, no study which had directly compared the rate of blood lactate decline in trained and untrained subjects had found any effect of training on the rate of blood lactate decline (15, 52), although some studies had provided indirect evidence which implied that this should be the case (57, 155).

Although it has been widely accepted that a greater rate of blood lactate decline during recovery from exercise reflects a more rapid rate of lactate removal (15, 52, 57, 59), the rate of blood lactate decline will not only be influenced by the rate at which lactate is removed from the blood, but also by the rate at which it is added to the blood by the previously active skeletal muscles. This being the case, a more rapid decline in blood lactate concentration during recovery from exercise may not necessarily be the result of a more rapid lactate removal, but could instead be due to a more rapid decrease in the rate at which lactate is added to the blood by the previously active skeletal

muscles. It has been an assumption of previous studies (15, 52, 57), including the work reported in Chapter 3, that the addition of lactate to the blood by previously active trained and untrained skeletal muscle decreases at the same rate during recovery. One of the aims of the work described in this chapter was to test the validity of this assumption.

A second aim of the work described in this chapter was to examine plasma FFA uptake by actively contracting trained and untrained skeletal muscle during incremental exercise in order to determine whether an increased plasma FFA uptake could contribute to a reduced lactate output by trained skeletal muscle during submaximal exercise. It is well recognised that endurance-training leads to an increased capacity for the utilisation of fats during exercise, but there is considerable debate relating to the source from which the additional FFA are derived. Some studies have indicated that the additional FFA are drawn from the plasma (81, 119, 200), citing evidence of an enhanced uptake of plasma FFA by trained skeletal muscle during exercise, whilst others have found either no difference (104) or a decrease (135) in plasma FFA utilisation following training, suggesting instead that intramuscular triacylglycerols are the source of the additional FFA utilised. The second purpose of the present study was therefore, to determine whether endurance-trained skeletal muscle takes up and utilises more plasma FFA than untrained skeletal muscle during exercise.

## 4.2 METHODS

**4.2.1 *Subjects and experimental conditions:*** Seven competitive male racquet sport players volunteered to participate in the study after providing informed consent.

**4.2.2 *Experimental design:*** Subjects attended the laboratory on three separate occasions. During the first visit to the laboratory subjects performed an incremental handgrip exercise test to familiarise themselves with this type of exercise (see General Methods, 2.4.2). During the second laboratory visit maximum handgrip strength (MHS), hand and forearm volumes and forearm circumferences were determined, and arterial and venous blood sampling catheters were inserted prior to subjects performing incremental handgrip exercise to exhaustion. Arterial and venous blood samples were taken at min five and ten of an initial ten minute rest period, during the final 15 sec of each work load during handgrip exercise, immediately upon the termination of exercise, and at 2.5, 5, 7.5, 10, 15, 20, 30 and 45 min post-exercise. Blood samples taken during the initial rest period and handgrip exercise were analysed for plasma glucose and FFA concentrations, whole blood lactate concentrations, blood gases, total hemoglobin concentration [tHb], percentage oxygen saturation (%$SO_2$), pH and haematocrit. Blood samples taken during recovery were not analysed for plasma glucose or FFA concentrations. Changes in plasma volume during handgrip exercise were calculated according to the method of Harrison (78). FBF was measured across each forearm throughout the protocol with the circulation to the hand left unoccluded (see General

Methods, 2.3.5b). Forearms with a peak oxygen uptake ($F\dot{A}VO_{2peak}$) greater than 100 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$ were classified as being endurance-trained, whilst those with values less than 100 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$ were classified as untrained. The untrained forearm was not always contralateral to the playing arm, but was in fact any forearm which demonstrated a peak oxygen uptake less than 100 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$. Due to technical problems venous blood samples from one trained forearm could not be obtained during recovery and all of the data for this arm was excluded from the study, leaving six trained (TRFA) and seven untrained forearms (UTFA).

Approximately two weeks after the second handgrip exercise test the peak systemic oxygen uptake ($\dot{V}O_{2peak}$) of each subject was determined during an incremental supine cycling test to volitional exhaustion (see General Methods, 2.4.3).

**4.2.3 *Blood lactate:*** Changes in the arterial blood lactate concentration and forearm net lactate output during recovery from handgrip exercise were modelled using the following biexponential time function:

$$La(t) = A_1(1-e^{-\gamma 1 t}) + A_2(1-e^{-\gamma 2 t}) + La(0)$$

where La($t$) was the arterial lactate concentration ($mmol \cdot l^{-1}$) or net lactate output ($\mu mol \cdot 100ml^{-1} \cdot min^{-1}$) at any *time* $t$, La(0) was the arterial lactate concentration ($mmol \cdot l^{-1}$) or net lactate output ($\mu mol \cdot 100ml^{-1} \cdot min^{-1}$) at the end of exercise, $A_1$

and $A_2$ were the amplitudes of the fitted exponential function for the arterial lactate concentration ($mmol \cdot l^{-1}$) or the net lactate output ($\mu mol \cdot 100ml^{-1} \cdot min^{-1}$), and $\gamma_1$ ($min^{-1}$) and $\gamma_2$ ($min^{-1}$) were rate constants (15, 56, 57, 59, 60, 155).

## 4.3 RESULTS

**4.3.1 *General:*** The anthropometric and physiological characteristics of the experimental subjects are reported in Table 4.1, and the characteristics of the UTFA and TRFA are presented in Table 4.2.

**Table 4.1.** ***Anthropometric and physiological characteristics of subjects (n=7).***

| | |
|---|---|
| Age (yr) | 25.3 ± 2.1 |
| Height (cm) | 182.3 ± 1.5 |
| Mass (kg) | 77.1 ± 2.3 |
| Systemic $\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 39.4 ± 5.1 |

Values are means ± SEM.

**Table 4.2.** ***Forearm characteristics***

| | Untrained forearms (n=7) | Trained forearms (n=6) |
|---|---|---|
| Circumference (cm) | 25.6 ± 0.5 | 26.9 ± 0.9 |
| Hand and forearm volume (ml) | 1988.6 ± 55.4 | 2206.7 ± 99.4 |
| MHS (kg) | 53.6 ± 2.2 | 62.2 ± 2.3* |
| FWC ($J \cdot 100ml^{-1}$) | 41.0 ± 4.0 | 67.7 ± 9.9* |
| $FA\dot{V}O_{2peak}$ ($\mu mol \cdot 100ml^{-1} \cdot min^{-1}$) | 88.2 ± 5.7 | 134.1 ± 14.1* |

FWC, Forearm work capacity; MHS, maximal handgrip strength; $FA\dot{V}O_{2peak}$, peak forearm oxygen uptake. Values are means ± SEM. * Significantly different from UTFA ($P<0.05$).

During handgrip exercise the TRFA reached higher absolute work loads than the UTFA prior to exhaustion (UTFA, 1.8 ± 0.1 W; TRFA 2.5 ± 0.1 W; $P<0.01$). Since there was no difference in hand and forearm volumes between the two groups, the higher work loads reached by the TRFA resulted in their completing more than 1.5 times as much work as the UTFA when expressed per unit volume of hand and forearm tissue (UTFA, 41.0 ± 4.0 Joules·$100ml^{-1}$; TRFA, 67.7 ± 9.9 Joules·$100ml^{-1}$). However, both groups of forearms reached fatigue at the same relative percentage of MHS (UTFA, 23.6 ± 1.4%; TRFA, 28.1 ± 2.6%).

**4.3.2 *Forearm blood flow:*** FBF during rest and incremental handgrip exercise is shown in Fig. 4.1. FBF was similar in both groups prior to handgrip exercise (UTFA, 2.1 ± 0.2 ml·100 $ml^{-1}$·$min^{-1}$; TRFA, 2.0 ± 0.1 ml·100 $ml^{-1}$·$min^{-1}$). With the onset of handgrip exercise FBF increased progressively in both groups of forearms, with no difference between the two groups at comparable work loads, or at the end of exercise, by which time FBF had reached 12.6 ± 1.0 ml·100 $ml^{-1}$·$min^{-1}$ in the UTFA, and 19.6 ± 3.4 ml·100 $ml^{-1}$·$min^{-1}$ in the TRFA.

FBF during recovery from handgrip exercise is shown in Fig. 4.2. Immediately post-exercise FBF increased rapidly in both groups of forearms, reaching peak values by 2.5 min of recovery (UTFA, 25.9 ± 2.6 ml·100 $ml^{-1}$·$min^{-1}$; TRFA, 26.7 ± 4.2 ml·100 $ml^{-1}$·$min^{-1}$; $P>0.05$), before declining rapidly during the rest of the recovery period. FBF had returned to pre-exercise values in both groups of forearms by the end of recovery.

**4.3.3 *Forearm oxygen uptake:*** Forearm oxygen uptake ($FA\dot{V}O_2$) during rest and incremental handgrip exercise is shown in Fig. 4.3. There was no difference in $FA\dot{V}O_2$ between the two groups of forearms prior to handgrip exercise (UTFA, 10.2 ± 1.1 µmol·100 $ml^{-1}$·$min^{-1}$; TRFA, 10.0 ± 0.8 µmol·100 $ml^{-1}$·$min^{-1}$). With the commencement of handgrip exercise $FA\dot{V}O_2$ increased progressively in both groups of forearms, with no difference in $FA\dot{V}O_2$ between the two groups at comparable work loads. However, significantly higher peak values for $FA\dot{V}O_2$ ($FA\dot{V}O_{2peak}$) were reached in the TRFA as a result of their being able to perform significantly higher work loads prior to

exhaustion (see Table 4.2). $F\dot{A}VO_2$ exhibited plateau-like behaviour in both groups of forearms at higher work loads, suggesting that maximal $F\dot{A}VO_2$ values had been reached.

$F\dot{A}VO_2$ during recovery from handgrip exercise is shown in Fig. 4.4. Immediately post-exercise, $F\dot{A}VO_2$ decreased rapidly, and had returned to pre-exercise values in both groups of forearms by the end of recovery.

**4.3.4 *Forearm respiratory exchange ratio:*** There was no difference in the forearm respiratory exchange ratio (FARER) between the two groups of forearms prior to handgrip exercise (UTFA, 0.61 ± 0.04; TRFA, 0.69 ± 0.05). During handgrip exercise, the FARER increased more rapidly in the UTFA ($P<0.05$), but reached similar values in both groups of forearms by the end of exercise (UTFA, 1.10 ± 0.13; TRFA, 1.11 ± 0.08).

**4.3.5 *Blood lactate:*** The venous blood lactate concentration during rest and incremental handgrip exercise is shown in Fig. 4.5. There was no difference in venous lactate concentration between the two groups of forearms immediately prior to handgrip exercise (UTFA, 0.66 ± 0.08 $mmol \cdot l^{-1}$; TRFA, 0.53 ± 0.07 $mmol \cdot l^{-1}$). During handgrip exercise, the venous lactate concentration increased in both groups, with the increase being more rapid in the UTFA such that the venous lactate concentration in the UTFA was significantly higher than in the TRFA at comparable submaximal work loads. By the end of exercise the

venous lactate concentration had reached similar values in both groups of forearms (UTFA, 3.81 ± 0.30 mmol·l$^{-1}$; TRFA, 3.06 ± 0.49 mmol·l$^{-1}$).

During recovery the venous blood lactate concentration exhibited a similar exponential decline in both groups of forearms, and had returned to pre-exercise values by the end of recovery (Fig. 4.6).

The arterial blood lactate concentration during rest and incremental handgrip exercise is shown in Fig. 4.7. Immediately prior to handgrip exercise there was no difference in arterial blood lactate concentration between the two groups of forearms (UTFA, 0.48 ± 0.05 mmol·l$^{-1}$; TRFA, 0.38 ± 0.07 mmol·l$^{-1}$). During handgrip exercise the arterial lactate concentration exhibited similar increases in both groups of forearms ($P<0.01$), reaching 0.78 ± 0.06 mmol·l$^{-1}$ in the UTFA and 0.66 ± 0.06 mmol·l$^{-1}$ in the TRFA by the end of exercise.

The arterial blood lactate concentration during recovery is shown in Fig. 4.8. Immediately post-exercise the arterial lactate concentration increased rapidly in both groups of forearms, reaching peak values by 2.5 min of recovery (UTFA, 0.86 ± 0.05 mmol·l$^{-1}$; TRFA, 0.73 ± 0.11 mmol·l$^{-1}$; $P>0.05$), before declining to reach pre-exercise values by the end of recovery. The parameters for the biexponential curve fit of the arterial lactate concentration during recovery are given in Table 4.3. The evolution of the arterial lactate concentration during the recovery period was well fit using the biexponential function, with the curve fit accounting for 88% of the observed variance for the UTFA and 96% for the TRFA, with no difference in the goodness-of-fit between

the two groups. There were no differences in any of the curve fitting parameters between the two groups of forearms.

**Table 4.3.** ***Parameters of biexponential curve fits to individual arterial lactate curves during recovery.***

| | Untrained forearms (n=7) | Trained forearms (n=6) |
|---|---|---|
| $A_1$ ($mmol \cdot l^{-1}$) | 0.122 ± 0.149 | 8.602 ± 6.857 |
| $\gamma 1$ ($min^{-1}$) | 4.898 ± 1.531 | 2.487 ± 1.367 |
| $A_2$ ($mmol \cdot l^{-1}$) | -50.955 ± 50.431 | -8.921 ± 6.841 |
| $\gamma 2$ ($min^{-1}$) | 0.063 ± 0.018 | 0.121 ± 0.047 |
| La(0) ($mmol \cdot l^{-1}$) | 0.775 ± 0.065 | 0.661 ± 0.060 |

$A_1$ and $A_2$, amplitudes of fitted exponential function; $\gamma 1$ and $\gamma 2$ velocity constants of fitted exponential function; La(0), arterial lactate concentration at end of exercise (*time* 0 of recovery).

The v-a blood lactate differences during rest and incremental handgrip exercise are shown in Fig. 4.9. There was a small positive v-a lactate difference in both groups of forearms prior to handgrip exercise (UTFA, 0.17 ± 0.05 $mmol \cdot l^{-1}$; TRFA, 0.15 ± 0.06 $mmol \cdot l^{-1}$) indicating net lactate output. The v-a lactate difference increased more rapidly in the UTFA during handgrip exercise, such that the v-a lactate difference in this group of forearms was greater than in the TRFA at comparable work loads. However, similar v-a lactate differences were reached in both groups of forearms by the end of exercise (UTFA, 3.03 ± 0.34 $mmol \cdot l^{-1}$; TRFA, 2.40 ± 0.49 $mmol \cdot l^{-1}$; $P>0.05$).

During recovery the v-a blood lactate difference declined at a similar rate in both groups of forearms, and had returned to pre-exercise values in both groups by the end of the recovery period (Fig. 4.10).

Net lactate flux across both groups of forearms during rest and incremental handgrip exercise is shown in Fig. 4.11. There was a small, but significant ($P<0.01$), net lactate output from both groups of forearms prior to handgrip exercise (UTFA, 0.30 ± 0.06 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$; TRFA 0.29 ± 0.13 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$). Net lactate output increased in both groups of forearms during handgrip exercise ($P<0.001$), with the increase being more rapid in the UTFA, such that they exhibited a significantly greater net lactate output than the TRFA at comparable work loads. However, by the end of exercise there was no difference in the rate of net lactate output between the two groups of forearms (UTFA, 37.37 ± 4.32 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$; TRFA, 43.23 ± 7.47 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$).

Net lactate flux across both groups of forearms during recovery is shown in Fig. 4.12. Net lactate output followed a similar pattern to the changes in FBF during recovery, increasing rapidly in both groups of forearms to reach similar peak values by 2.5 min post-exercise (UTFA, 62.43 ± 9.83 μmol·100 $ml^{-1}$·$min^{-1}$; TRFA, 53.57 ± 15.82 μmol·100 $ml^{-1}$·$min^{-1}$; $P>0.05$), before declining to pre-exercise values by the end of the recovery period. The pattern of net lactate output during recovery was well described using a biexponential time function, the parameters for the curve fit of which are given in Table 4.4. The curve fit accounted for 99.1% of the observed variance for the UTFA and 99.7% for the TRFA, with no difference in the goodness-of-fit between the two groups. There were no differences in any of the curve fitting parameters between the two groups of forearms during recovery, and net lactate output was more highly correlated with FBF (UTFA, $r = 0.97$; TRFA, $r = 0.94$) than with the v-a lactate difference (UTFA, $r = 0.86$; TRFA, $r = 0.89$) in the UTFA ($P<0.01$), but not in the TRFA ($P>0.05$) during this period.

**Table 4.4.** ***Parameters of biexponential curve fits to individual net lactate output curves during recovery.***

| | Untrained forearms (n=7) | Trained forearms (n=6) |
|---|---|---|
| $A_1$ ($\mu mol \cdot 100ml^{-1} \cdot min^{-1}$) | 3884.943 ± 1902.006 | 105.097 ± 48.125 |
| $\gamma 1$ ($min^{-1}$) | 5.066 ± 2.315 | 6.022 ± 1.866 |
| $A_2$ ($\mu mol \cdot 100ml^{-1} \cdot min^{-1}$) | -3921.450 ± 1901.633 | -147.204 ± 47.724 |
| $\gamma 2$ ($min^{-1}$) | 0.385 ± 0.069 | 0.329 ± 0.031 |
| La(0) ($\mu mol \cdot 100ml^{-1} \cdot min^{-1}$) | 37.371 ± 4.323 | 43.232 ± 7.473 |

$A_1$ and $A_2$, amplitudes of fitted exponential function; $\gamma 1$ and $\gamma 2$ velocity constants of fitted exponential function; La(0), net lactate output at end of exercise (*time* 0 of recovery).

**4.3.6** ***Plasma volume changes:*** The arterial plasma volume did not change significantly during handgrip exercise in either group of forearms. The venous plasma volume on the other hand, decreased in both groups of forearms during handgrip exercise, decreasing by 6.1 ± 1.2% in the UTFA and 5.1 ± 2.5% in the TRFA by the end of exercise, these values not being significantly different (Fig. 4.13).

**4.3.7 *Plasma free fatty acids:*** Arterial plasma FFA concentrations during rest and incremental handgrip exercise are shown in Fig. 4.14. There was no difference in the arterial plasma FFA concentration between the two groups prior to handgrip exercise (UTFA, 0.76 ± 0.06 $mmol \cdot l^{-1}$; TRFA, 0.62 ± 0.16 $mmol \cdot l^{-1}$). During handgrip exercise the arterial plasma FFA concentration exhibited a small increase in both groups of forearms, with no difference in concentration between the two groups at comparable work loads, or at the end of exercise (UTFA, 0.97 ± 0.13 $mmol \cdot l^{-1}$; TRFA, 0.93 ± 0.25 $mmol \cdot l^{-1}$).

There was no difference in venous plasma FFA concentration between the two groups prior to handgrip exercise (UTFA, 0.72 ± 0.13 $mmol \cdot l^{-1}$; TRFA, 0.53 ± 0.13 $mmol \cdot l^{-1}$). During handgrip exercise the venous plasma FFA concentration did not change significantly from pre-exercise values in either group of forearms, even when corrected for the decreases in venous plasma volume.

The v-a plasma FFA difference was similar in both groups of forearms prior to handgrip exercise (UTFA, -0.04 ± 0.08 $mmol \cdot l^{-1}$; TRFA, -0.10 ± 0.08 $mmol \cdot l^{-1}$), and did not change significantly from these values in either group of forearms during handgrip exercise.

Net forearm plasma FFA flux during rest and incremental handgrip exercise is shown in Fig. 4.15. There was no significant net uptake or ouptut of plasma FFA by either group of forearms prior to handgrip exercise (UTFA, -0.04 ± 0.08 $\mu mol.100ml^{-1}.min^{-1}$; TRFA, -0.13 ± 0.10 $\mu mol.100ml^{-1}.min^{-1}$). During

handgrip exercise both groups of forearms began to take up plasma FFA on a net basis, with no difference in the rate of uptake between the two groups at comparable work loads. Peak rates of plasma FFA uptake were reached prior to the end of exercise in both groups of forearms, with no difference in the rate of peak uptake between the two groups (UTFA, -0.75 ± 0.13 $\mu mol.100ml^{-1}.min^{-1}$; TRFA, -1.08 ± 0.37 $\mu mol.100ml^{-1}.min^{-1}$).

**4.3.8 *Plasma glucose:*** The arterial plasma glucose concentration was similar in both groups of forearms prior to handgrip exercise (UTFA, 5.36 ± 0.11 $mmol \cdot l^{-1}$; TRFA, 5.70 ± 0.23 $mmol \cdot l^{-1}$). During handgrip exercise the arterial plasma glucose concentration did not change significantly from pre-exercise values in either group of forearms.

The venous plasma glucose concentration during rest and incremental handgrip exercise is shown in Fig. 4.16. There was no difference in the venous plasma glucose concentration between the two groups of forearms prior to handgrip exercise (UTFA, 5.01 ± 0.14 $mmol \cdot l^{-1}$; TRFA, 5.28 ± 0.22 $mmol \cdot l^{-1}$). During handgrip exercise the venous plasma glucose concentration increased in both groups of forearms, with no difference in concentration between the two groups at comparable work loads, or at the end of exercise (UTFA, 5.31 ± 0.14 $mmol \cdot l^{-1}$; TRFA 5.61 ± 0.25 $mmol \cdot l^{-1}$). The increases in venous plasma glucose concentration during handgrip exercise could be accounted for by the decrease in venous plasma volume since, once corrected for the decreases in venous plasma volume, the venous plasma glucose concentration did not change from pre-exercise values in either group of forearms during handgrip exercise.

The v-a plasma glucose difference was similar in both groups of forearms prior to handgrip exercise (UTFA, -0.35 ± 0.07 $mmol \cdot l^{-1}$; TRFA, -0.42 ± 0.10 $mmol \cdot l^{-1}$), and did not change significantly from these values in either group of forearms during handgrip exercise.

Both groups of forearms exhibited a net uptake of plasma glucose prior to handgrip exercise (UTFA, -0.44 ± 0.10 $\mu mol.100ml^{-1}.min^{-1}$; TRFA, -0.47 ± 0.16 $\mu mol.100ml^{-1}.min^{-1}$) which did not change significantly in either group of forearms during handgrip exercise.

## 4.4 DISCUSSION

The principal findings of this study were that the kinetics of the addition of lactate to the blood during recovery from handgrip exercise were similar for both TRFA and UTFA, and that the reduced net lactate output by the TRFA during submaximal handgrip exercise was not due to a greater uptake of plasma FFA. These findings suggest that the more rapid decline in blood lactate concentration during recovery from whole body exercise reported for endurance-trained subjects in Chapter 3 was most likely due to an increased rate of lactate removal, and that reduced rates of lactate production by endurance-trained skeletal muscle during submaximal exercise do not result from an increased utilisation of plasma FFA.

In Chapter 3 it was found that the blood lactate concentration decreased more rapidly in endurance-trained subjects than in untrained subjects during recovery from supine cycling exercise. This finding was interpreted, as it has been suggested in the literature (15, 52, 57, 155), as reflecting a more rapid rate of lactate removal in the trained subjects. However, such an interpretation could only be valid if training does not influence the kinetics of lactate output by skeletal muscle during recovery from exercise. An increased rate of decline in the addition of lactate to the blood by previously active endurance-trained skeletal muscle could result in a more rapid decline in blood lactate concentration just as easily as an increase in the rate of blood lactate removal. However, the present study found no difference in the kinetics of the addition of lactate to the blood between trained and untrained skeletal muscle during recovery, suggesting that the increased rate of blood lactate decline found in the endurance-trained subjects in Chapter 3 was most likely the result of an increased rate of lactate removal.

Upon the cessation of handgrip exercise there was a rapid increase in FBF in both groups of forearms, presumably as a result of the release of mechanical compression of the blood vessels within the actively contracting forearm muscles. A transient post-exercise hyperaemia such as this would assist in maintaining a more favourable gradient for the movement of lactate from the previously active muscle to the blood, thereby serving to reduce muscle lactate concentrations more rapidly and hasten recovery from fatigue. It was of interest to note that the rate of net forearm lactate output during recovery was

more highly correlated with FBF ($r = 0.97$) than with the v-a lactate difference ($r = 0.86$) in the UTFA ($P<0.01$), but not in the TRFA (FBF, $r = 0.94$; v-a diff, $r = 0.89$; $P>0.05$), suggesting that the TRFA were less reliant on an increased blood flow to maintain an outwardly directed gradient for lactate translocation. It has recently been shown that training can increase the capacity to transport lactate across the sarcolemma by increasing the number and affinity of lactate transporter proteins (162). The sarcolemmal lactate transporter content of the TRFA in the present study may have been greater than in the UTFA, thus accounting for the reduced reliance on blood flow to achieve a given rate of net lactate output during recovery. Alternatively, a redistribution of blood flow within the muscles of the TRFA, whereby the same total blood flow was distributed through a more extensive capillary network (95), could have providing a slower transit time and larger surface area for lactate movement, thereby requiring less of an increase in blood flow to achieve a given rate of lactate output. Regardless of the differing importance of an increase in blood flow between trained and untrained skeletal muscle in terms of helping to achieve a given rate of lactate output during recovery from exercise, the hyperaemia observed in both groups of forearms upon the cessation of handgrip exercise in the present study would facilitate lactate output in both groups of forearms, thereby hastening recovery.

During handgrip exercise the rate of net plasma glucose uptake by the active forearms was similar for both groups at comparable work loads, yet lactate output was significantly lower in the TRFA. This suggests either that the glycogenolytic rate was activated more slowly in the TRFA during exercise, or

that a greater proportion of carbohydrate (glucose and glycogen) was directed into the Krebs cycle for oxidation, with less being available for reduction to lactate. The former is more likely since, if a greater proportion of carbohydrate was directed into the Krebs cycle for oxidation in the TRFA the FARER would have increased more rapidly than in the UTFA as the exercise intensity increased and more and more carbohydrate was oxidised. This was not the case, the FARER increased more rapidly in the UTFA during handgrip exercise, indicating a more rapid increase in the oxidation of carbohydrate in these arms compared with the TRFA. Nevertheless, although a more rapid rise in FARER in the UTFA during handgrip exercise suggests a more rapid increase in carbohydrate utilisation, the calculations of FARER used in the present study were unlikely to reflect steady-state values due to the relatively short work periods employed (ie. 1 min 45 sec work periods). Calculations of FARER made under non-steady state conditions do not necessarily reflect the metabolic status of a tissue since the values can be considerably influenced by formation of $CO_2$ from the buffering of $H^+$ by the bicarbonate system. Nevertheless, given that other studies have shown muscle glycogen stores to be depleted less in trained skeletal muscle during exercise (53, 99, 113) a slower activation of glycogenolysis would seem the most likely explanation for the reduced net lactate output by the TRFA during handgrip exercise in the present study.

Previous studies which have examined the effect of training on the uptake of plasma FFA by skeletal muscle during exercise have reported a greater uptake (119, 200) and oxidation (200) of plasma FFA by trained muscle. In the present study, no difference was found in plasma FFA uptake between

the endurance-trained and untrained forearms. The difference in findings between the present study and previous studies which have reported an increased plasma FFA uptake (119, 200) could be due to the previous studies having all examined plasma FFA uptake during prolonged submaximal exercise. In each of these previous studies the exercise lasted for 2-3 hours and plasma FFA uptake by the trained skeletal muscles was only greater during the latter stages of exercise. No explanations were offered in those studies as to why plasma FFA uptake was only greater in trained skeletal muscle near the end of exercise, however, it is possible that in the present study no difference was found in plasma FFA uptake between the UTFA and the TRFA due to the relatively short duration of the exercise protocol (ie. ~20 min). Since there was no difference in the rate of plasma FFA uptake between the two groups of forearms at comparable work loads any increased fat utilisation by the TRFA must have been fuelled by fatty acids derived from some source other than the plasma. Intramuscular triacylglycerols would be the most likely source since the activity of type L hormone-sensitive lipase, the enzyme thought to be responsible for triacylglycerol lipolysis in skeletal muscle (152) is elevated in trained skeletal muscle (153), and intramuscular triacylglycerols have been shown to be depleted more in trained than in untrained skeletal muscle during exercise (99). Nonetheless, the possibility of a greater contribution of plasma triacylglycerols to fatty acid oxidation in trained skeletal muscle cannot be ruled out since some studies have found no increased contribution from intramuscular triacylglycerols in trained skeletal muscle during exercise, despite finding evidence of an increased rate of FFA oxidation (119).

In conclusion, the data from the present study indicate that there is no difference in the kinetics of the addition of lactate to the blood between previously active trained and untrained skeletal muscle during recovery from exercise. This suggests that the more rapid rate of decline in the arterial blood lactate concentration of the trained subjects reported in Chapter 3 was most likely due to an increased rate of lactate removal in tissues other than the exercising muscle. Furthermore, the finding of a reduced net lactate output at a given work load in the TRFA during handgrip exercise, despite no difference in net plasma FFA uptake between the two groups, suggests that if additional FFA utilisation by trained skeletal muscle leads to a reduced net lactate production during exercise it must be the result of an increased oxidation of FFA derived from some source other than the plasma.

## 4.5 FIGURES

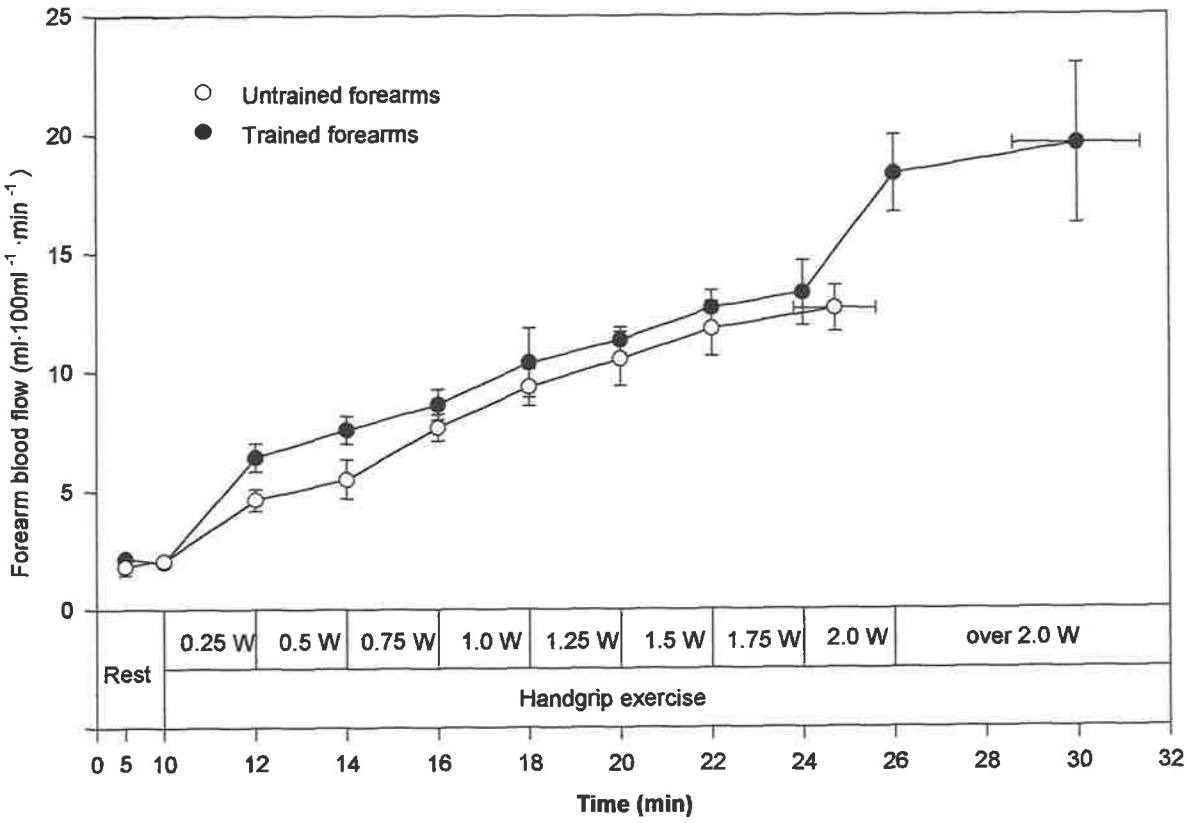


Fig 4.1. Forearm blood flow in endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

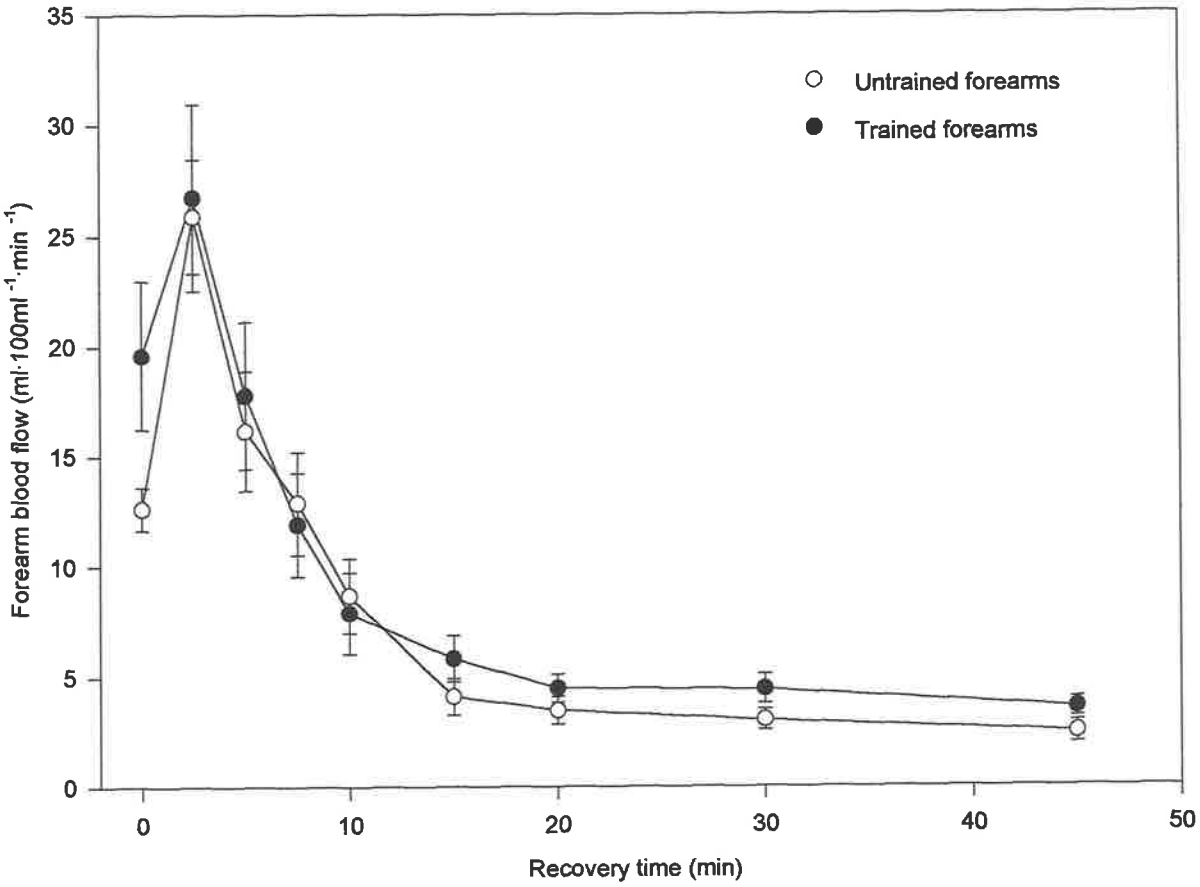


Fig 4.2. Forearm blood flow in endurance-trained (n=6) and untrained (n=7) forearms during recovery from handgrip exercise. Values are means ± SEM.

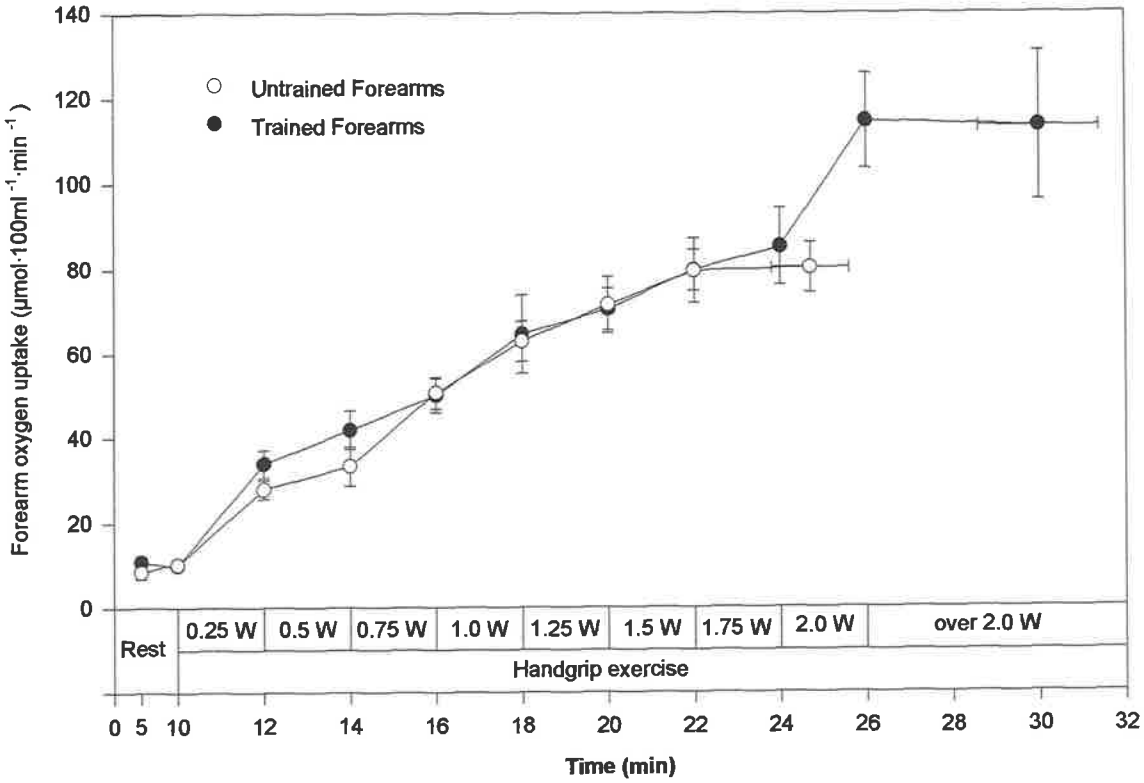


Fig. 4.3. Forearm oxygen uptake in endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

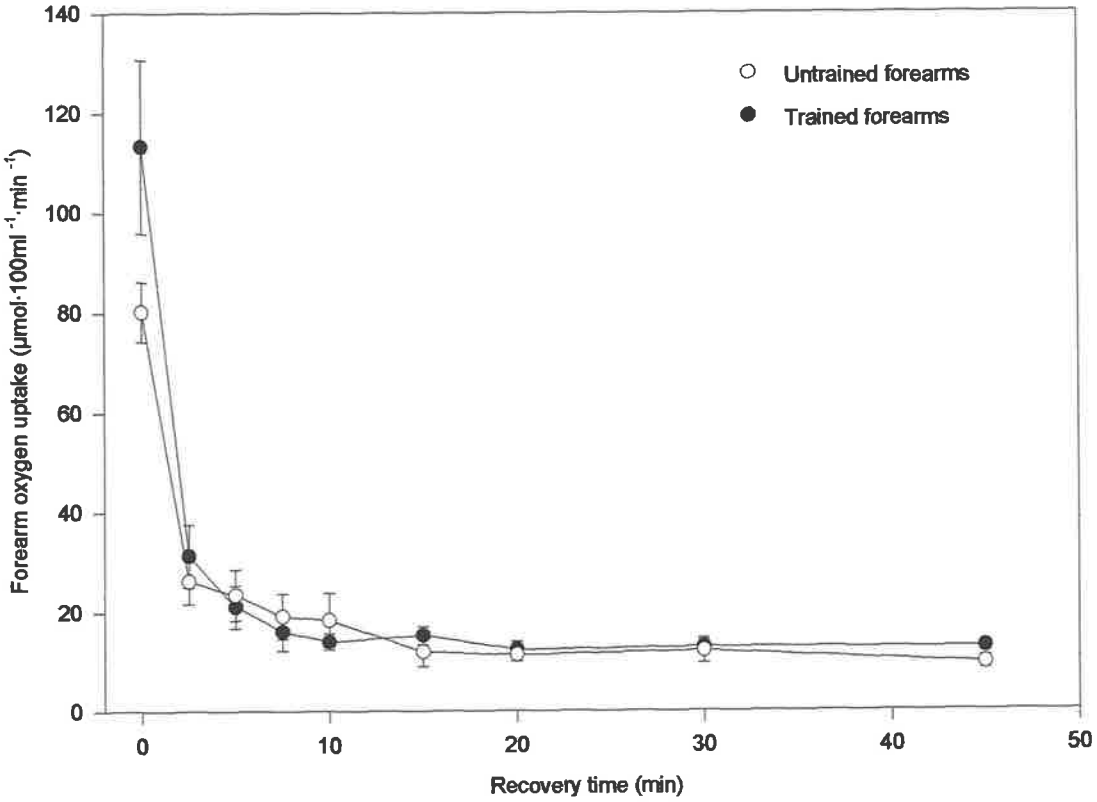


Fig. 4.4. Forearm oxygen uptake in endurance-trained (n=6) and untrained (n=7) forearms during recovery from handgrip exercise. Values are means ± SEM.

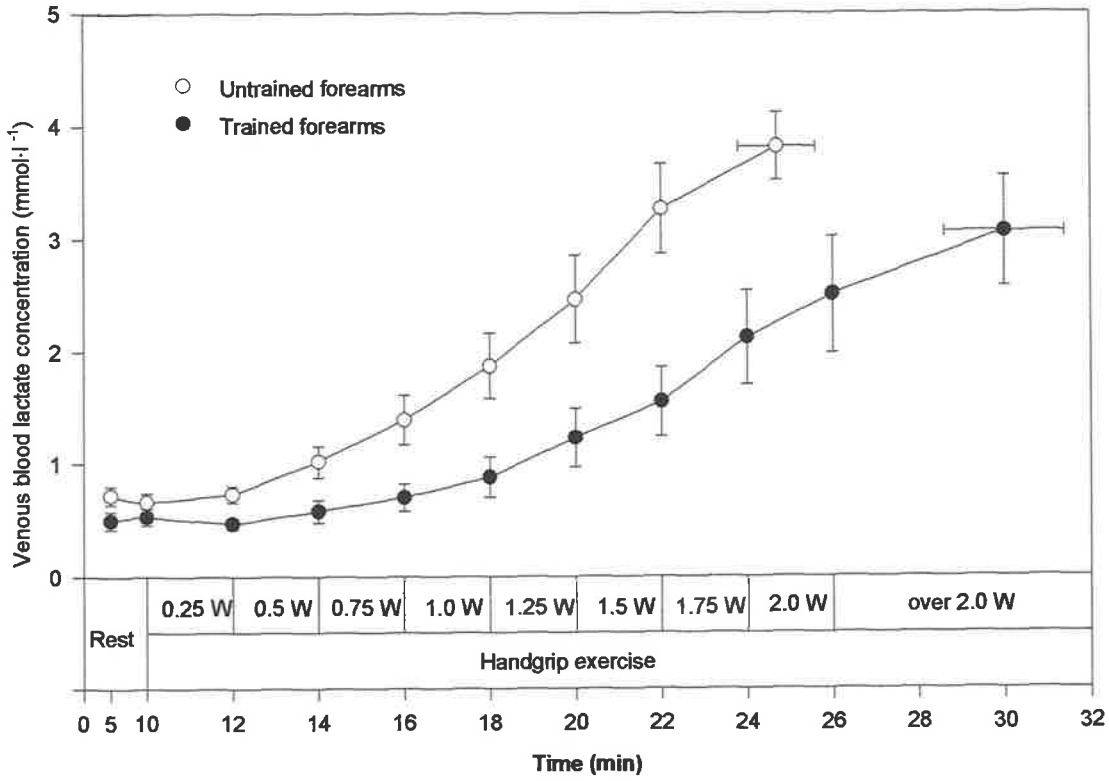


Fig. 4.5. Venous blood lactate concentrations in endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

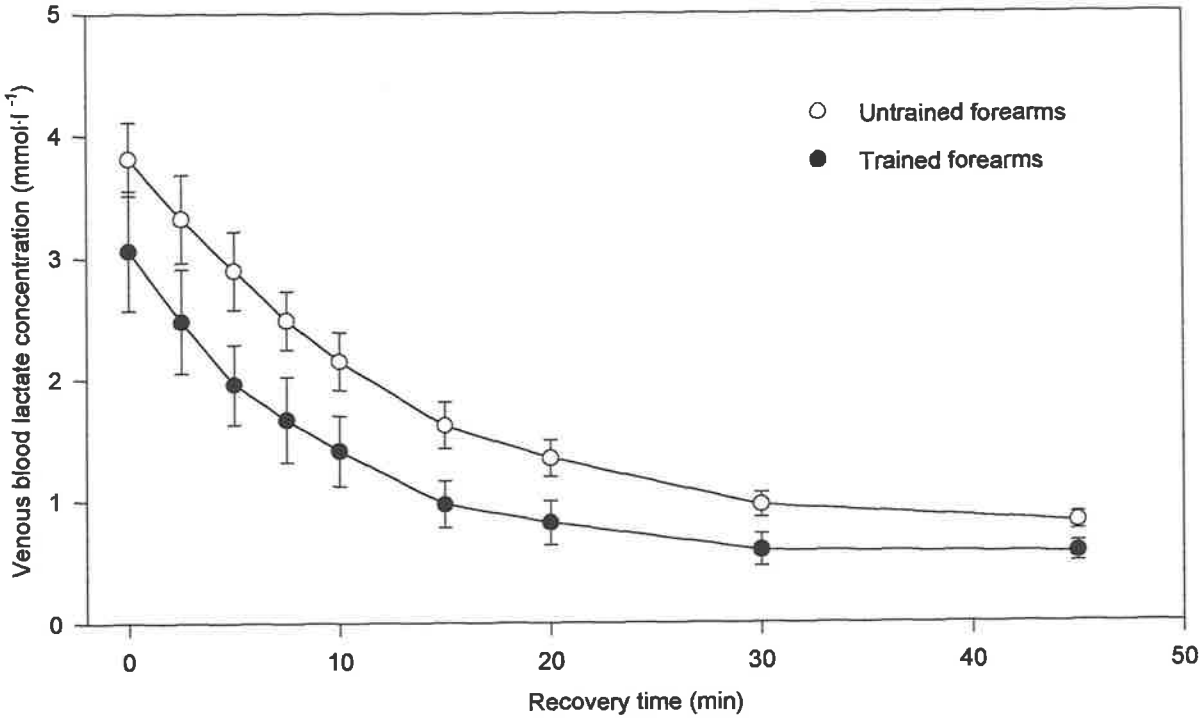


Fig. 4.6. Venous blood lactate concentrations in endurance-trained (n=6) and untrained (n=7) forearms during recovery from incremental handgrip exercise. Values are means ± SEM.

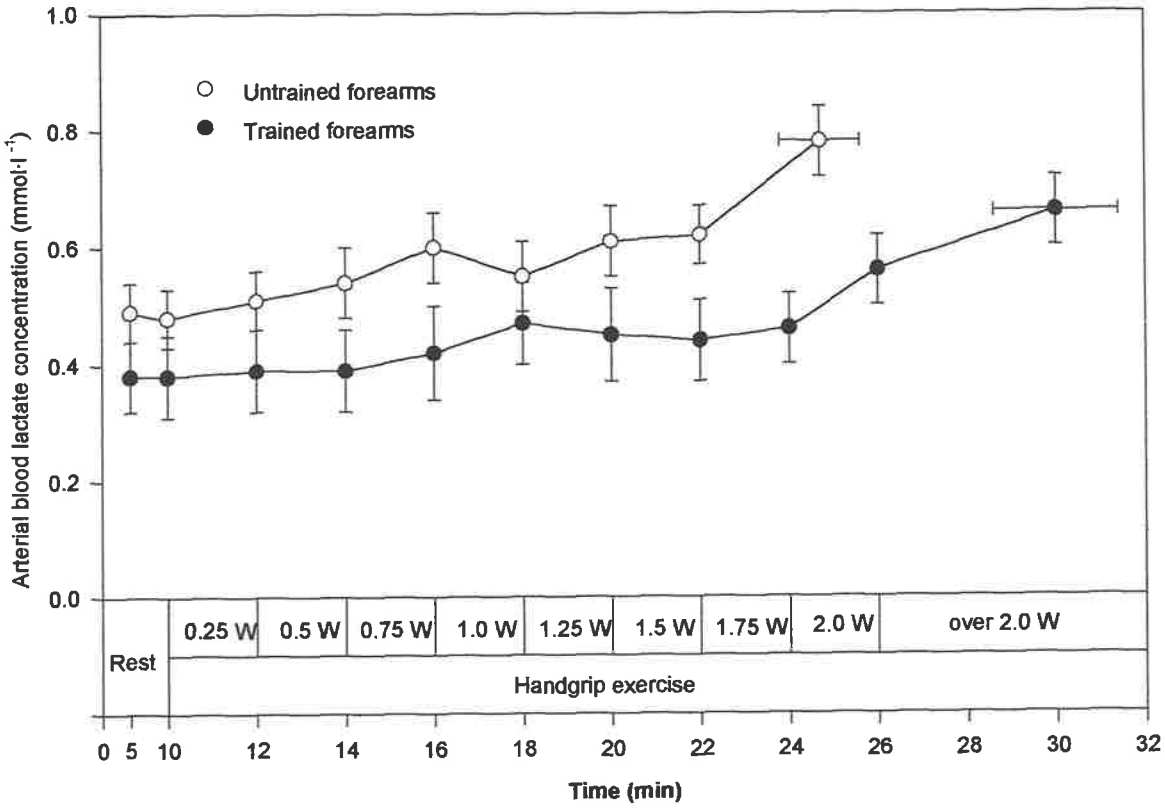


Fig. 4.7. Arterial blood lactate concentrations in endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

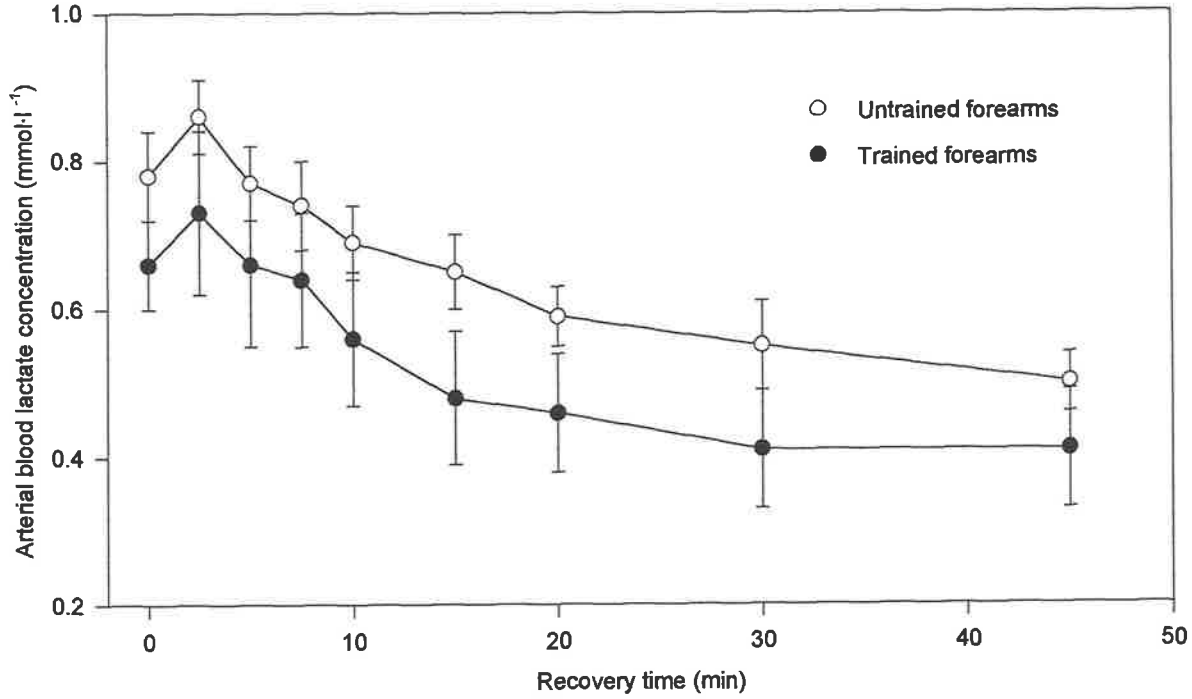


Fig 4.8. Arterial blood lactate concentrations in endurance-trained (n=6) and untrained (n=7) forearms during recovery from incremental handgrip exercise. Values are means ± SEM.

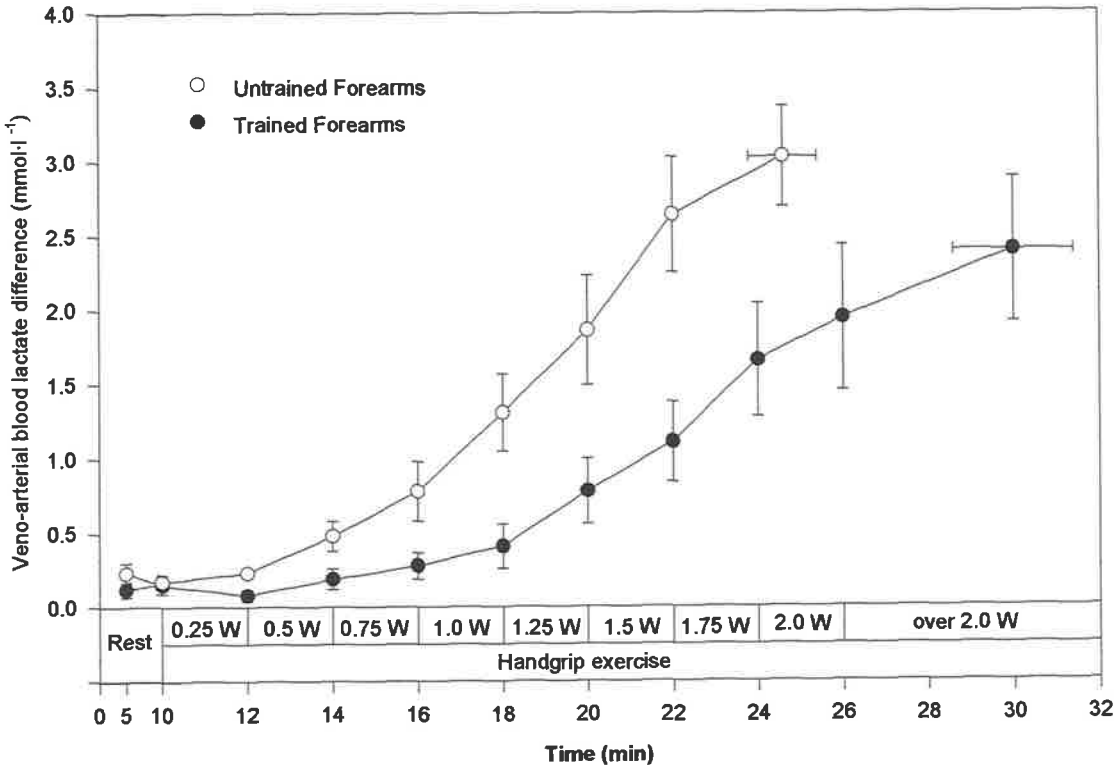


Fig. 4.9. Veno-arterial blood lactate difference across endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

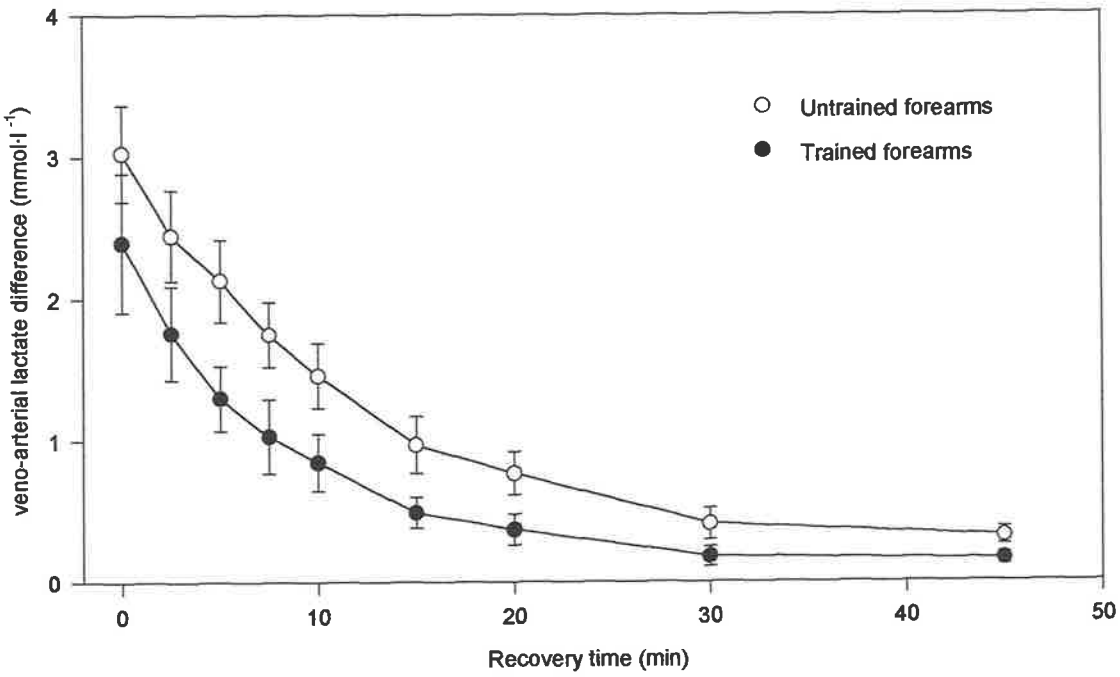


Fig. 4.10. Veno-arterial blood lactate differences in endurance-trained (n=6) and untrained (n=7) forearms during recovery from incremental handgrip exercise. Values are means ± SEM.

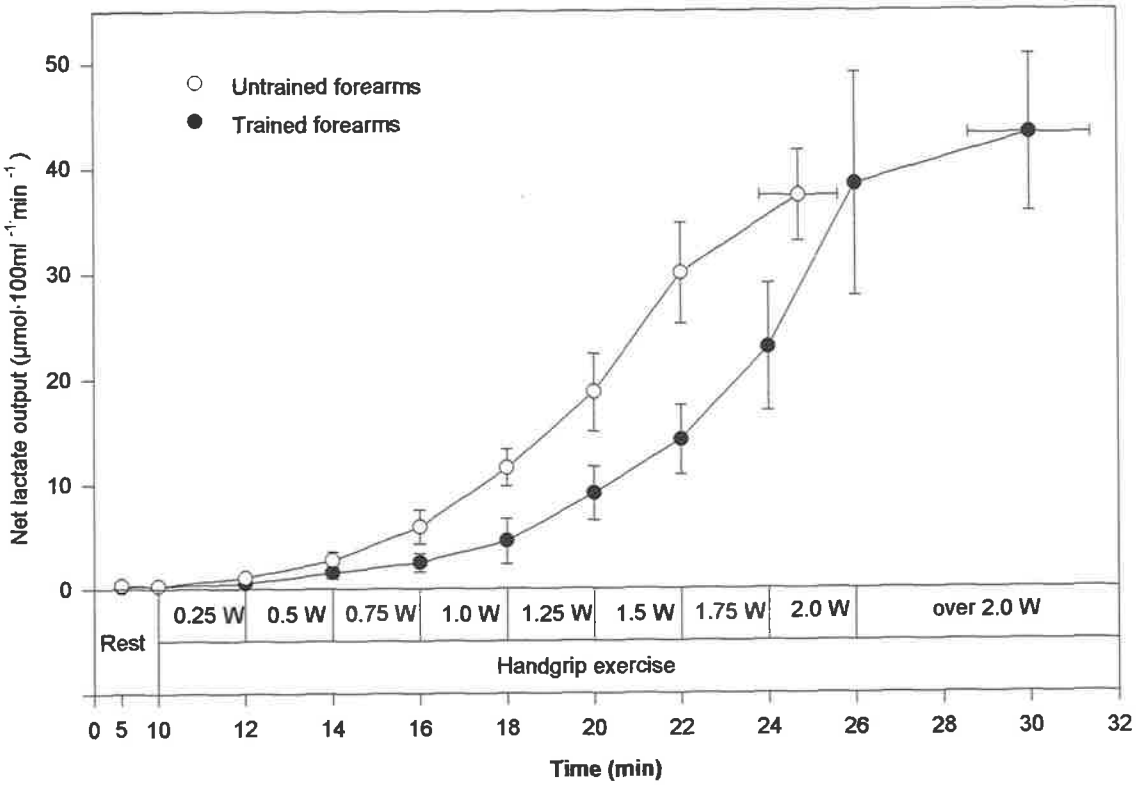


Fig. 4.11. Net lactate output by endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

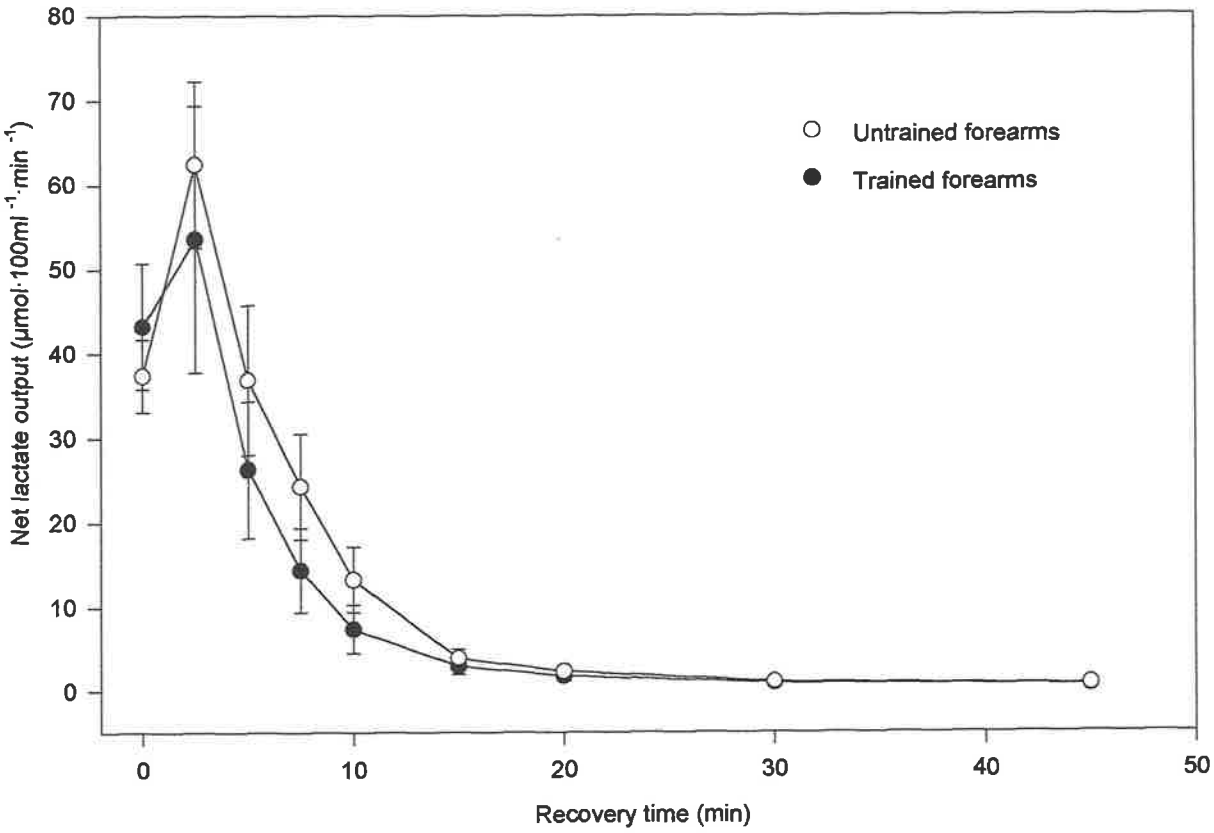


Fig. 4.12. Net lactate output by endurance-trained (n=6) and untrained (n=7) forearms during recovery from incremental handgrip exercise. Values are means ± SEM.

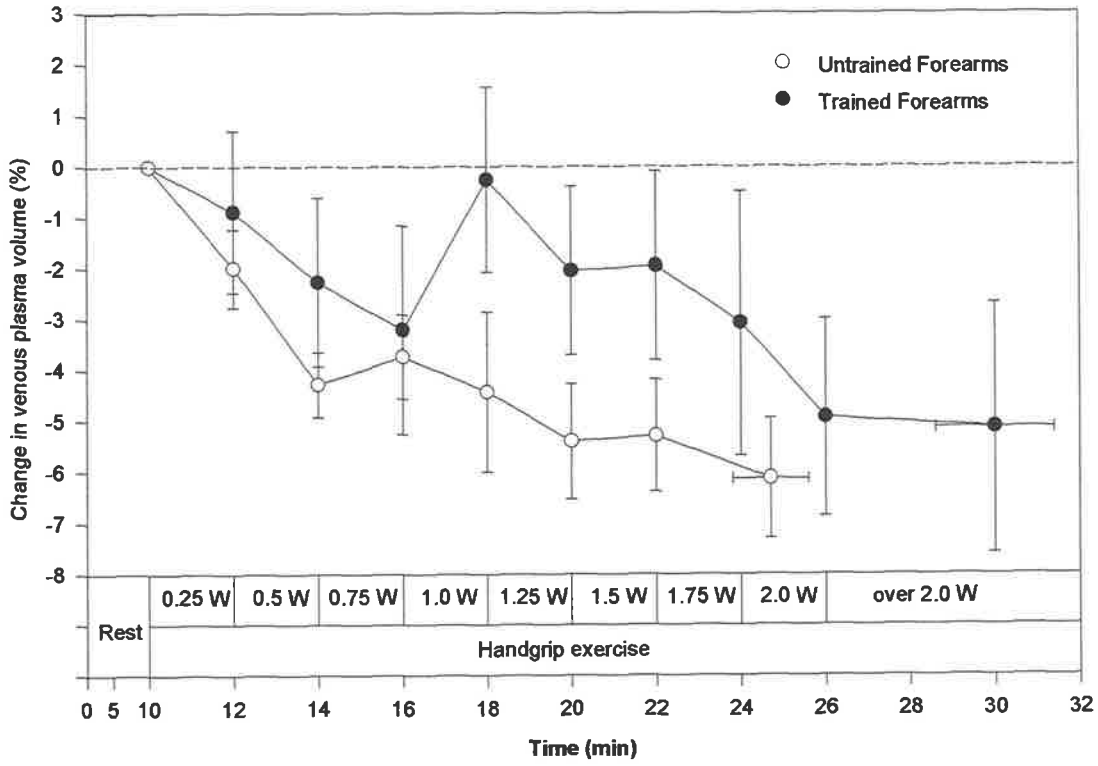


Fig. 4.13. Percentage changes in venous plasma volume during incremental handgrip exercise in endurance-trained (n=6) and untrained (n=7) forearms. Values are means ± SEM.

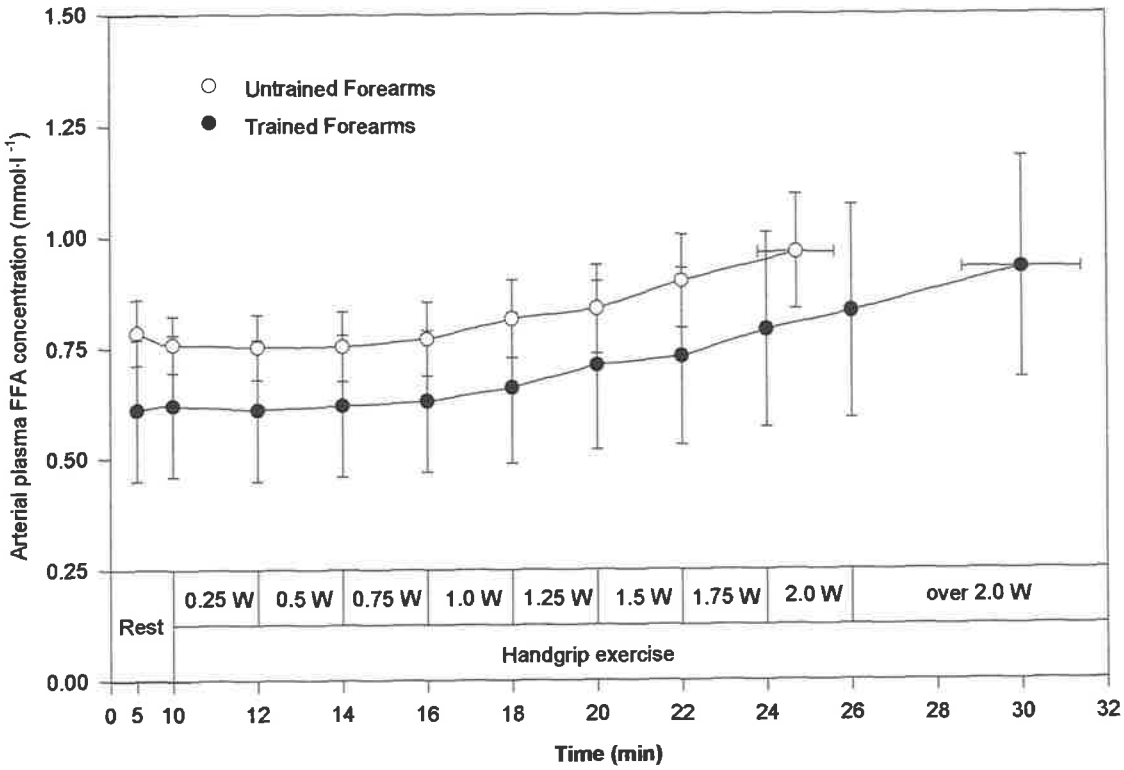


Fig. 4.14. Concentration of plasma FFA in arterial blood perfusing endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

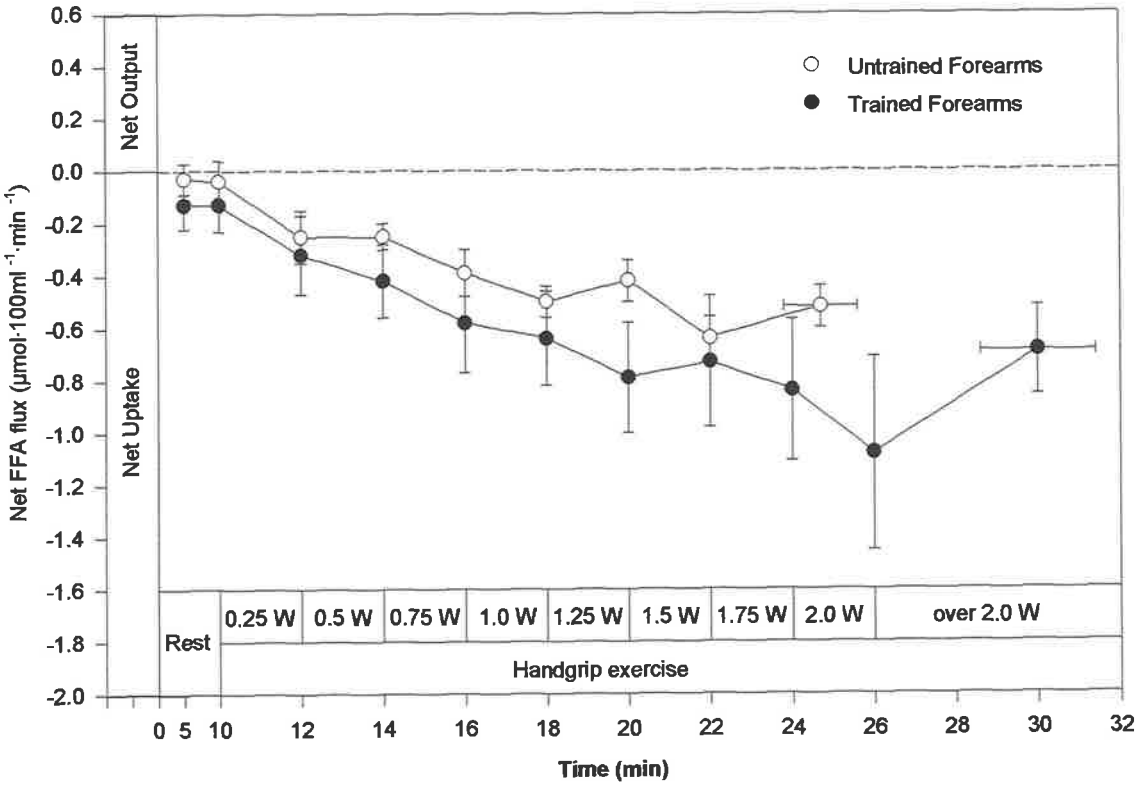


Fig 4.15. Net plasma FFA uptake by endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

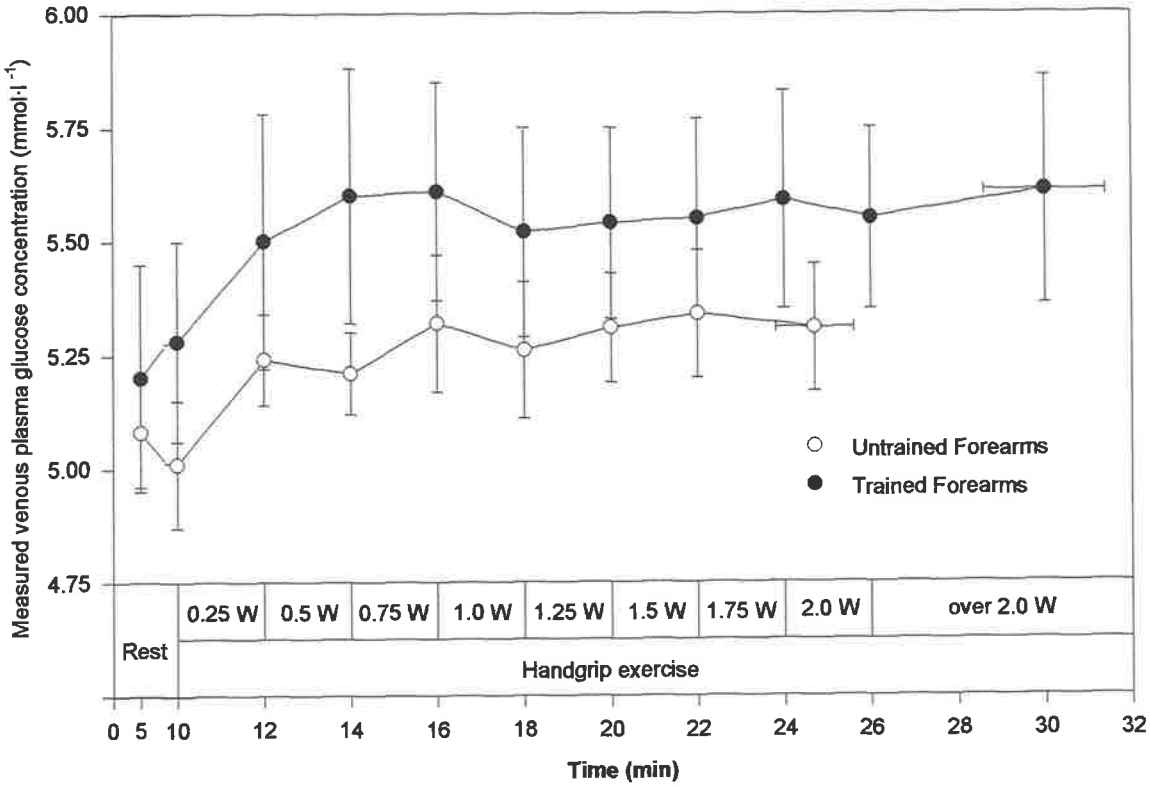


Fig. 4.16. Venous plasma glucose concentrations in endurance-trained (n=6) and untrained (n=7) forearms during rest and incremental handgrip exercise. Values are means ± SEM.

# CHAPTER 5 - LOCAL TRAINING EFFECTS ON LACTATE REMOVAL BY SKELETAL MUSCLE

## 5.1 INTRODUCTION

The work described in Chapters 3 and 4 of this thesis, as well as the findings of numerous other studies (43-45, 132, 159, 179, 180, 193, 195) indicate that endurance training increases the rate at which lactate can be removed from the blood. It has been suggested that the more rapid lactate removal may be the result of an increase in lactate removal by trained skeletal muscle (44, 45, 64). Endurance training has been shown to reduce total lactate dehydrogenase (LDH) activity in skeletal muscle, with an increase in the proportion of the heart specific isoenzyme (LDH-H) (184), and to increase the enzymes of the malate-aspartate shuttle (179). These adaptations could potentially increase the rate at which skeletal muscle can oxidise NADH and convert lactate to pyruvate, resulting in a more rapid utilisation of lactate.

A recent study carried out in this laboratory (28), which examined lactate removal by inactive trained and untrained forearm skeletal muscle during supine cycling exercise, found no difference in lactate removal between trained and untrained skeletal muscle. However, this previous study examined differences in lactate removal during near maximal exercise, when arterial blood lactate concentrations were relatively high (~11 $mmol \cdot l^{-1}$) and, given that lactate removal

is a saturable process (4, 44, 49, 193), it is possible that no difference was found due to lactate removal being saturated in both trained and untrained skeletal muscle.

The aim of the present study was to examine lactate removal by endurance-trained and untrained skeletal muscle over a range of arterial blood lactate concentrations to determine whether endurance-trained skeletal muscle removes more lactate from the blood than untrained skeletal muscle. Incremental sodium lactate infusion was used to elevate the arterial blood lactate concentration in an effort to avoid changes in cutaneous blood flow which occur during exercise, and to reduce variability in the blood lactate concentrations.

## 5.2 METHODS

**5.2.1 *Subjects and experimental conditions:*** Six competitive male racquet sport players volunteered to participate in the study after providing informed consent.

**5.2.2 *Experimental design:*** Subjects attended the laboratory on three separate occasions. On each occasion forearm circumferences and volumes were measured. During the first visit subjects performed incremental handgrip exercise to fatigue in order to familiarise themselves with this type of exercise. Fatigue

was defined as the inability to successfully complete two contractions in succession. During the second visit to the laboratory maximal handgrip strength (MHS) was determined before arterial and venous catheters were inserted into the brachial artery of one arm and the median antecubital veins of both arms. Subjects then performed incremental handgrip exercise to fatigue whilst FBF was measured, and arterial and venous blood samples were taken for determination of blood lactate concentrations. Forearms were categorised as being either endurance-trained or untrained according to the relative amount of work performed by each arm ($J \cdot 100ml^{-1}$). Those forearms which performed more than 46 $J \cdot 100ml^{-1}$ were classified as being endurance-trained (TRFA), whilst those which were unable to perform more than 42 $J \cdot 100ml^{-1}$ were designated as being untrained (UTFA). Based on this selection criterion, the data for one forearm had to be excluded, leaving six TRFA and five UTFA. On the final visit to the laboratory each subject received an incremental venous infusion of sodium lactate. FBF was measured in each forearm and arterial and venous blood samples were taken at min 5 and 10 of an initial rest period prior to infusion, as well as at selected time intervals during and post-infusion. Blood samples were analysed for haematocrit, hemoglobin concentration, blood gases, blood lactate and pH. Percentage changes in plasma volume were calculated according to the method of Harrison (78), with the blood sample obtained at the end of the initial 10 min rest period being used as the point of reference.

**5.2.3 *Sodium lactate infusion:*** For the infusion of sodium lactate a 14G Drum-Cartridge Catheter (Venisystems) was inserted into the basilic vein at the elbow of the arm which did not contain the brachial artery catheter. The infusion catheter was advanced approximately 30 cm towards the heart to ensure that its tip was located proximal to the venous collecting cuff used for the measurement of FBF. This was done to ensure that the sodium lactate infusate solution would mix freely with the systemic circulation when the venous collecting cuff was inflated during blood flow measurements. Following an initial 10 min rest period, during which basal measurements were recorded, an incremental infusion of sodium lactate was delivered using a Harvard infusion pump (Model 945). Infusion rates were normalised for body mass, with the initial rate of infusion being 0.01 $ml \cdot kg^{-1} \cdot min^{-1}$. This was increased by a further 0.01 $ml \cdot kg^{-1} \cdot min^{-1}$ every three min up to a maximal rate of 0.06 $ml \cdot kg^{-1} \cdot min^{-1}$. The infusion pump was calibrated prior to each trial and infusion rates were checked by comparing the actual volume of sodium lactate solution infused during each trial with the desired infusion volume.

## 5.3 RESULTS

**5.3.1 *General:*** The characteristics of the subjects grouped on the basis of forearm training status are presented in Table 5.1, and the characteristics of the forearms themselves are presented in Table 5.2.

***Table 5.1. Characteristics of subjects grouped according to forearm training status.***

| | Untrained forearms (n=5) | Trained forearms (n=6) |
|---|---|---|
| Age (yr) | 19.2 ± 0.2 | 25.0 ± 2.0* |
| Mass (kg) | 71.8 ± 1.2 | 79.5 ± 2.7* |
| Height (cm) | 178.8 ± 0.7 | 185.9 ± 1.8* |
| Supine cycling $\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 38.5 ± 1.8 | 47.6 ± 6.2 |

Values are means ± SEM. * Significant difference between groups $P < 0.05$)

***Table 5.2. Forearm descriptive characteristics.***

| | Untrained forearms (n=5) | Trained forearms (n=6) |
|---|---|---|
| Forearm Girth (cm) | 24.8 ± 0.5 | 27.3 ± 0.4* |
| Hand and forearm Volume (ml) | 1884.0 ± 48.7 | 2180.0 ± 76.4* |
| Forearm volume (ml) | 1512.0 ± 38.8 | 1773.3 ± 50.5* |
| Maximum Handgrip Strength (kg) | 49.1 ± 3.1 | 57.8 ± 2.9 |

Values are means ± SEM. * Significantly different from untrained forearms $P < 0.05$)

**5.3.2 *Forearm work parameters during handgrip exercise:*** The UTFA reached fatigue at 1.7 ± 0.1 W, whilst the TRFA were able to exercise up to a work load of 2.5 ± 0.2 W ($P<0.01$) before reaching fatigue. Despite the TRFA being significantly larger than the UTFA (see Table 5.2), the higher work loads reached by the TRFA still resulted in their performing almost twice as much work as the UTFA when expressed per unit volume of hand and forearm tissue (UTFA, 35.1 ± 2.3 Joules·100 $ml^{-1}$; TRFA, 66.1 ± 9.5 Joules·100 $ml^{-1}$).

**5.3.3 *Blood lactate during handgrip exercise:*** Changes in the arterial blood lactate concentration during rest and incremental handgrip exercise are shown in Fig. 5.1. The arterial blood lactate concentration was slightly lower in the TRFA than in the UTFA prior to handgrip exercise (UTFA, 0.54 ± 0.03 mmol·$l^{-1}$; TRFA, 0.35 ± 0.07 mmol·$l^{-1}$). During handgrip exercise there were small parallel increases in the arterial lactate concentration in both groups of forearms, such that the concentration remained slightly lower in the TRFA at comparable work loads. However, by the end of handgrip exercise there was no significant difference in the arterial lactate concentration between the two groups (UTFA, 0.86 ± 0.06 mmol·$l^{-1}$; TRFA, 0.67 ± 0.06 mmol·$l^{-1}$; $P>0.05$).

Changes in the venous blood lactate concentration during rest and incremental handgrip exercise are shown in Fig. 5.2. Like the arterial blood lactate concentration, the venous blood lactate concentration was slightly lower in the TRFA than in the UTFA prior to handgrip exercise (UTFA, 0.75 ± 0.07

$mmol \cdot l^{-1}$; TRFA, 0.49 ± 0.07 $mmol \cdot l^{-1}$). During handgrip exercise the venous blood lactate concentration increased in both groups of forearms ($P<0.001$), but the rate of increase was more rapid in the UTFA, such that the difference in resting venous lactate concentrations became greater during exercise. However, by the end of handgrip exercise there was no significant difference in venous blood lactate concentration between the two groups (UTFA, 4.12 ± 0.55 $mmol \cdot l^{-1}$; TRFA, 2.99 ± 0.42 $mmol \cdot l^{-1}$; $P>0.05$).

The veno-arterial blood lactate differences during rest and incremental handgrip exercise are shown in Fig 5.3. There were positive v-a lactate differences in both groups of forearms prior to handgrip exercise (UTFA, 0.21 ± 0.05 $mmol \cdot l^{-1}$; TRFA, 0.14 ± 0.06 $mmol \cdot l^{-1}$; $P>0.05$), indicating net forearm lactate output. The v-a lactate difference increased in both groups of forearms during handgrip exercise ($P<0.001$), but was significantly lower in the TRFA at comparable work loads. However, v-a lactate differences were similar in both groups of forearms by the end of exercise (UTFA, 3.26 ± 0.58 $mmol \cdot l^{-1}$; TRFA, 2.33 ± 0.42 $mmol \cdot l^{-1}$; $P>0.05$).

Net forearm lactate flux during rest and incremental handgrip exercise is shown in Fig 5.4. There was a small net lactate output by both groups of forearms prior to handgrip exercise (UTFA, 0.34 ± 0.05 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$; TRFA, 0.29 ± 0.14 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$). During handgrip exercise the rate of net lactate output increased in both groups of forearms ($P<0.001$), but was

significantly lower in the TRFA at comparable work loads. However, by the end of exercise the difference in rates of net lactate output between the two groups of forearms had disappeared (UTFA, 41.04 ± 9.07 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$; TRFA, 32.91 ± 5.31 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$; $P>0.05$).

**5.3.4 *Sodium lactate infusion:*** During sodium lactate infusion, the absolute volumes of solution infused (UTFA, 44.2 ± 0.3 ml; TRFA, 50.0 ± 1.1 ml) were not significantly different from the desired infusion volumes (UTFA, 44.1 ± 0.0 ml; TRFA, 49.8 ± 1.2 ml), and amounted to 100.3 ± 0.8 % and 100.4 ± 0.3 % of the desired volumes respectively. Relative volumes of 0.62 ± 0.01 $ml \cdot kg^{-1}$ and 0.63 ± 0.01 $ml \cdot kg^{-1}$ of infusate solution were infused into the subjects with UTFA and TRFA respectively, which corresponded to the infusion of 2.47 ± 0.06 $mmol \cdot kg^{-1}$ and 2.52 ± 0.05 $mmol \cdot kg^{-1}$ of sodium lactate, these values being not significantly different.

**5.3.5 *Forearm blood flow during sodium lactate infusion:*** FBF was not different between the two groups of forearms prior to sodium lactate infusion (UTFA, 1.3 ± 0.1 $ml \cdot 100\ ml^{-1} \cdot min^{-1}$; TRFA, 2.1 ± 0.4 $ml \cdot 100\ ml^{-1} \cdot min^{-1}$), and FBF did not change significantly from these values during either sodium lactate infusion or recovery.

**5.3.6 *Blood lactate during sodium lactate infusion:*** The arterial blood lactate concentration during rest, sodium lactate infusion and recovery is shown in Fig. 5.5. There was no significant difference in the arterial blood lactate concentration between the two groups of forearms prior to sodium lactate infusion (UTFA, 0.87 ± 0.12 mmol·l$^{-1}$; TRFA, 0.69 ± 0.08 mmol·l$^{-1}$). During infusion, the arterial blood lactate concentration increased in both groups of forearms, but was significantly higher in the blood perfusing the UTFA, reaching peak values of 6.2 ± 0.5 mmol·l$^{-1}$ and 5.6 ± 0.2 mmol·l$^{-1}$ in the UTFA and TRFA respectively by the end of infusion. The relationship between the infusion rate ($R_{inf}$) and the arterial blood lactate concentration was better described ($P<0.001$) by an exponential function of the form $Ca(R_{inf}) = Ae^{-bRinf} + C$, where $A$, $b$ and $C$ are constants ($r^2 = 0.988 \pm 0.004$) than by simple linear regression ($r^2 = 0.972 \pm 0.006$). During recovery there was no difference in arterial lactate concentration between the two groups, with the arterial lactate concentration declining exponentially to reach pre-infusion values in both groups by the end of the recovery period.

The venous blood lactate concentration during rest, sodium lactate infusion and recovery is shown in Fig. 5.6. The concentration of lactate in the venous blood draining the TRFA was significantly lower than in that draining the UTFA prior to sodium lactate infusion (UTFA, 1.14 ± 0.16 mmol·l$^{-1}$; TRFA, 0.74 ± 0.04 mmol·l$^{-1}$). During infusion the venous lactate concentration exhibited a parallel increase in both groups of forearms, such that the venous lactate concentration remained significantly lower in the TRFA. The venous blood

lactate concentration reached peak values 6.0 ± 3.7 min post-infusion in the UTFA (3.4 ± 0.3 mmol·l$^{-1}$) and 5.0 ± 2.0 min post-infusion in the TRFA (2.7 ± 0.2 mmol·l$^{-1}$), with no difference in the time to peak, but with the peak values being significantly lower in the TRFA. After having reached these peak values, the venous blood lactate concentration declined exponentially in both groups of forearms, and was significantly lower in the blood draining the TRFA. By the end of the 120 min recovery period the venous lactate concentration was still slightly elevated above preinfusion values in the blood draining the TRFA (1.02 ± 0.03 mmol·l$^{-1}$), but was not different from preinfusion values in the UTFA (1.47 ± 0.21 mmol·l$^{-1}$).

The veno-arterial blood lactate differences during rest, incremental sodium lactate infusion and recovery are shown in Fig 5.7. There was a positive v-a lactate difference in both groups of forearms prior to infusion (UTFA, 0.27 ± 0.11 mmol·l$^{-1}$; TRFA, 0.07 ± 0.06 mmol·l$^{-1}$; $P>0.05$). During infusion the v-a lactate difference quickly became negative in both groups of forearms indicating net forearm lactate uptake, with peak v-a differences being reached at the end of the infusion period (UTFA, -2.99 ± 0.57 mmol·l$^{-1}$; TRFA -3.27 ± 0.17 mmol·l$^{-1}$; $P>0.05$). Post-infusion, the v-a lactate difference decreased rapidly and had returned to preinfusion values in both groups of forearms by the end of recovery.

Net forearm lactate flux during rest, sodium lactate infusion and recovery is shown in Fig. 5.8. There was no difference in net forearm lactate flux between the two groups prior to lactate infusion (UTFA, 0.38 ± 0.15 μmol·100 $ml^{-1}$·$min^{-1}$; TRFA, 0.07 ± 0.10 μmol·100 $ml^{-1}$·$min^{-1}$). During lactate infusion both groups of forearms began to take up lactate on a net basis. The rate of uptake was linearly related to the arterial blood lactate concentration (UTFA, r = -0.94; TRFA, r = -0.96) (see Fig. 5.9), but varied independently of FBF (UTFA, r = -0.44; TRFA, r = -0.21; P>0.05). There were no differences in the slope (UTFA, -1.1 ± 0.2; TRFA, -1.1 ± 0.1) or y-intercept values (UTFA, 1.3 ± 0.5; TRFA, 0.7 ± 0.1) for the regression equations describing this relationship, so the data were pooled to give an overall correlation coefficient of r=-0.84 (P<0.001), and a regression equation with a slope of -1.1 and y-intercept of 1.1.

During infusion there was no difference in the total arterial lactate loads delivered to each group of forearms (UTFA, 91.17 ± 11.75 μmol·100 $ml^{-1}$; TRFA, 78.60 ± 11.79 μmol·100 $ml^{-1}$). Of these loads the UTFA took up 41.50 ± 7.59 μmol·100 $ml^{-1}$ and the TRFA 41.46 ± 5.88 μmol·100 $ml^{-1}$, which amounted to 45.40 ± 4.11% and 53.25 ± 2.10% of the respective arterial loads, with no difference between the two groups. During recovery, the quantities of lactate released into the circulation by each group of forearms (UTFA, 53.79 ± 31.32 μmol·100 $ml^{-1}$; TRFA, 26.29 ± 21.06 μmol·100 $ml^{-1}$) were not significantly different from the quantities taken up during infusion, such that there was no significant

total net lactate production or disposal (UTFA, 9.57 ± 34.42 μmol·100 $ml^{-1}$; TRFA, -15.17 ± 20.97 μmol·100 $ml^{-1}$) in either group of forearms.

***5.3.7 Forearm oxygen uptake, carbon dioxide output and respiratory exchange ratio during sodium lactate infusion:*** $FA\dot{V}O_2$ was similar in both groups of forearms prior to sodium lactate infusion (UTFA, 4.90 ± 0.92 μmol·100 $ml^{-1}$·$min^{-1}$; TRFA, 5.96 ± 0.53 μmol·100 $ml^{-1}$·$min^{-1}$) and did not vary significantly from these values during infusion or recovery. Similarly, there was no difference in forearm carbon dioxide output ($FA\dot{V}CO_2$) (UTFA, 3.04 ± 0.71 μmol·100 $ml^{-1}$·$min^{-1}$; TRFA, 3.19 ± 0.64 μmol·100 $ml^{-1}$·$min^{-1}$) or FARER (UTFA, 0.62 ± 0.06; TRFA, 0.55 ± 0.12) between the two groups of forearms prior to infusion, and neither of these parameters changed significantly during infusion or recovery.

***5.3.8 Plasma volume changes during sodium lactate infusion:*** Plasma volume changes during sodium lactate infusion and recovery are shown in Fig. 5.10. The infusion of sodium lactate resulted in rapid increases in arterial and venous plasma volumes in both groups of forearms ($P<0.001$). The arterial plasma volume expanded by 16.2 ± 1.4 % in the UTFA and 13.9 ± 1.5% in the TRFA, with these values being not significantly different. The venous plasma volume expanded at the same rate as the arterial plasma volume in the TRFA, but in the UTFA the initial expansion of the venous plasma volume was more

rapid than the arterial plasma volume. Arterial and venous plasma volumes remained significantly elevated above preinfusion values in both groups of forearms throughout the recovery period.

**5.3.9 *Mean arterial blood pressure during sodium lactate infusion:*** Prior to sodium lactate infusion there was no difference in MAP between the two groups (UTFA, 86.4 ± 1.7 mmHg; TRFA, 96.5 ± 2.7 mmHg). During lactate infusion MAP increased at a similar rate in both groups, reaching 103.2 ± 4.0 mmHg in the UTFA and 109.8 ± 1.5 mmHg in the TRFA by the end of infusion, these values being not significantly different. MAP was significantly correlated with the changes in arterial plasma volume during infusion ($r=0.80$, $P<0.05$).

**5.3.10 *Acid-base changes during sodium lactate infusion:*** Arterial pH at rest, during sodium lactate infusion and recovery is shown in Fig. 5.11. There was no difference in arterial pH between the two groups of forearms prior to lactate infusion (UTFA, 7.407 ± 0.003; TRFA, 7.409 ± 0.006). During infusion arterial pH increased at a similar rate in both groups of forearms, reaching peak values of 7.472 ± 0.011 in the UTFA and 7.473 ± 0.011 in the TRFA by the end of the infusion period. During recovery the arterial pH remained elevated above pre-infusion values in both groups of forearms, but was significantly higher in the TRFA group.

Venous pH at rest, during sodium lactate infusion and recovery is shown in Fig. 5.12. There was no difference in venous pH between the two groups of forearms prior to infusion (UTFA, 7.351 ± 0.004; TRFA, 7.356 ± 0.005). During infusion venous pH increased at a similar rate in both groups of forearms, reaching 7.385 ± 0.004 in the UTFA and 7.387 ± 0.010 in the TRFA by the end of the infusion period. Venous pH continued to increase in both groups of forearms after infusion had ceased, reaching peak values of 7.413 ± 0.005 and 7.430 ± 0.003 by 64.5 ± 19.0 and 69.2 ± 19.5 min post-infusion in the UTFA and TRFA respectively. There was no difference in the time to peak venous pH between the two groups, but the peak values were significantly higher in the TRFA.

## 5.4 DISCUSSION

The principal finding of the present study was that inactive endurance-trained skeletal muscle does not have an increased capacity for net lactate uptake and/or disposal in comparison to inactive untrained skeletal muscle. The TRFA demonstrated a greater work capacity (UTFA, 35.1 ± 2.3 Joules·100 $ml^{-1}$; TRFA, 66.1 ± 9.5 Joules·100 $ml^{-1}$), and lower net lactate output than the UTFA during handgrip exercise, pointing to significant adaptations to endurance exercise, but during lactate infusion there was no difference in lactate uptake between the two groups, and neither group of forearms demonstrated any significant net metabolic lactate disposal.

Donovan and Pagliassotti (44, 45) reported that resting trained rats maintained lower arterial blood lactate concentrations than resting untrained rats during incremental lactate infusion. This ability to maintain lower blood lactate concentrations in the trained rats during infusion was attributed to greater rates of oxidation and gluconeogenic cycling of lactate, with the authors proposing that inactive trained skeletal muscle was the site of the increased lactate oxidation. However, the results of the present study suggest that this is not the case. An increased rate of lactate oxidation by trained skeletal muscle should increase the blood-muscle lactate gradient and facilitate lactate uptake at a given arterial blood lactate concentration. In the present study, the rate of lactate uptake by the inactive forearm skeletal muscles was linearly related to the arterial lactate concentration in both groups of forearms (UTFA, $r = -0.94$ ; TRFA, $r = -0.96$) and there was no difference in the rate of net lactate uptake between the two groups at a given arterial lactate concentration. This suggests that the rate of lactate uptake by skeletal muscle is a function of the concentration of lactate in the arterial blood perfusing the forearm and is not influenced by the training status of the arm. The finding of no difference in the rate of net forearm lactate uptake at a given arterial blood lactate also indicates, albeit indirectly, that there was no difference in net lactate oxidation between the two groups of forearms since, if the TRFA had oxidised more lactate than the UTFA, a greater rate of forearm lactate uptake would have been expected due to an increased blood-muscle lactate gradient. Further evidence, based on estimates of net lactate disposal,

indicated that, quite apart from there being no difference in net lactate disposal between the two groups of forearms, neither group of forearms demonstrated any significant net lactate disposal at all, with all of the lactate taken up by both groups of forearms being taken up passively. Given these findings, it is unlikely that trained skeletal muscle was responsible for the increased lactate oxidation reported in trained rats by Donovan and Pagliassotti (44, 45).

A tissue which could have been responsible for the increased lactate oxidation in trained rats reported by Donovan and Pagliassotti (44, 45) is the heart. The heart is a net consumer of lactate both at rest and during exercise (192), and there is some evidence that myocardial lactate extraction is increased through training (118). However, there is no evidence from the present study with which to either confirm or refute this hypothesis. Apart from an increased rate of lactate oxidation, Donovan and Pagliassotti (44, 45) also reported an increased removal of lactate via gluconeogenesis in trained rats. The tissue responsible for this increased gluconeogenesis was not identified by the authors but, based on current information, it would seem most likely that the kidneys were the site of the increased gluconeogenic activity. Few adaptations to endurance training have been noted in hepatic gluconeogenic enzymes (212) making an increased gluconeogenic removal of lactate by the liver unlikely, but elevated glucose production from lactate in slices of kidney cortex from trained animals has been reported (125). Furthermore, the kidneys can receive up to 25% of cardiac output at rest (198), and a relatively high blood flow combined with an increased

gluconeogenic capacity following training make the kidneys the most likely site for an increased removal of lactate via gluconeogenesis.

The present finding of no significant net lactate disposal in either group of forearms during lactate infusion agrees with the finding of a similar study by Catcheside and Scroop (31). Catcheside and Scroop (31) examined lactate removal by inactive forearm skeletal muscle during supine cycling exercise and found that all of the lactate removed by the inactive forearms could be accounted for by passive uptake, with no metabolic disposal. Nevertheless, the present finding differs from that of another study (28) in which inactive forearms disposed of approximately 87% of the lactate taken up during leg exercise. The different findings of these studies in relation to the metabolic disposal of lactate by inactive skeletal muscle may relate to differences in the concentrations of lactate in the arterial blood perfusing the skeletal muscle. In the present study, and that of Catcheside and Scroop (31), where no significant metabolic disposal of lactate occurred, the arterial lactate concentrations reached approximately 5 and 6 $mmol \cdot l^{-1}$ respectively, whereas in the study which reported significant net lactate disposal (28), the arterial lactate concentration reached ~11 $mmol \cdot l^{-1}$. The rate of lactate uptake by inactive skeletal muscle has previously been shown to be related to the arterial blood lactate concentration (71), as was the case in the present study (UTFA, $r = -0.94$ ; TRFA, $r = -0.96$), and since the lactate dehydrogenase (LDH) reaction is a near equilibrium reaction (150), an increase in lactate uptake could lead to an increase in net lactate disposal via mass action.

However, because the predominant LDH isozyme in skeletal muscle is LDH-M, which favours net lactate production (41), the rate of net lactate uptake would need to be sufficient to raise the sarcoplasmic lactate concentration enough to drive the LDH-M reaction in reverse in order for net lactate disposal to occur. The 5 $mmol \cdot l^{-1}$ arterial lactate concentration reached in the present study, and the 6 $mmol \cdot l^{-1}$ in the study by Catcheside and Scroop (31) may not have been sufficient to achieve this, thereby resulting in no net metabolic lactate disposal, whereas the concentration of 11 $mmol \cdot l^{-1}$ achieved in the study which did report significant metabolic lactate disposal may have been.

Studies using isotopic tracer techniques have indicated that active skeletal muscle can simultaneously release lactate into, and remove lactate from, the blood (194). According to the lactate shuttle hypothesis (23), this simultaneous release and removal of lactate is the result of lactate produced by some less oxidative fibres within an active skeletal muscle being taken up and oxidised by other fibres within the muscle which have a higher oxidative capacity. It seems possible therefore, that the reduced net lactate output by the endurance-trained forearms during handgrip exercise in the present study could have been due to more of the lactate produced by lesser trained skeletal muscle fibres being taken up and oxidised by fibres which had adapted to training. This would lead to a subsequent reduction in the amount of lactate appearing in the venous blood draining the active muscle. However, given that no difference was found in lactate removal between the two groups of forearms during lactate infusion, it

seems more likely that the reduced net lactate output by the trained forearms during handgrip exercise was a function of a reduction in net lactate production. This seems particularly so when one also considers that glycogen depletion (53) and carbohydrate utilisation (14, 165, 177) are reduced in trained skeletal muscle during exercise.

Previous studies which have used lactate infusions to elevate blood lactate concentrations have reported increases in both systemic (114) and skeletal muscle (4) $\dot{V}O_2$, with the increased $\dot{V}O_2$ being attributed to increased oxidation of the infused lactate (4, 114). In the present study systemic $\dot{V}O_2$ was not measured during infusion, but sodium lactate infusion had no effect on $FA\dot{V}O_2$. It is possible that $FA\dot{V}O_2$ did not increase during sodium lactate infusion in the present study because the forearms did not dispose of any significant quantity of lactate. However, Chin et al., (32) and Buckley et al., (28) found no increase in $\dot{V}O_2$ of inactive skeletal muscle during lactate infusion and exercise respectively, despite significant net lactate disposal. Given that some studies have found a relationship between lactate disposal and increases in $\dot{V}O_2$ (4, 114), whilst others have not (28, 32), tends to suggest that any such increases in $\dot{V}O_2$ are not directly related to the metabolic disposal of lactate.

Infusion of the sodium lactate solution resulted in significant expansions of both arterial and venous plasma volumes. The expansion of plasma volume would have resulted from the high osmolality of the infusate solution (4 Osm) increasing the osmotic pressure of the blood. As the blood traversed the various tissue beds the increased osmotic pressure would have drawn fluid from the tissues, leading to the expanded plasma volume. The finding of a significant correlation between the increase in arterial plasma volume and MAP ($r=0.80$, $P<0.05$) suggests that autoregulation of blood pressure was unable to compensate for the large expansion in plasma volume, leading to significant increases in MAP. The venous plasma volume expanded at the same rate as the arterial plasma volume in the TRFA, but in the UTFA the expansion of the venous plasma volume was initially more rapid than the arterial expansion, indicating that as the arterial blood traversed the UTFA water was drawn into the intravascular space. The reason why the UTFA lost water to the vascular space, whilst the TRFA did not, is unclear, but it is possible that the increased muscle glycogen (67, 68) and mitochondrial protein (13, 93, 146, 180) content of trained skeletal muscle enabled the binding of more water molecules, thereby helping to preserve the intramuscular water content and preventing the loss of intramuscular fluid to the vascular compartment. Nevertheless, regardless of the mechanism, it appears that there may be some adaptation to training which helps the muscle to protect its water content.

Despite the relative rates of lactate infusion being the same in all subjects the concentration of lactate was significantly lower in the blood perfusing the TRFA during the infusion period. This may reflect differences in the training status of the subjects whose arms made up the two groups since, despite there being no statistical difference in peak $\dot{V}O_2$ between the subjects when grouped according to forearm training status (see Table 5.1.), there was a trend for the mean values to be slightly higher for the TRFA group. This trend may be indicative of a training effect since, although there was no statistical difference in peak $\dot{V}O_2$ between the two groups, it has been shown that metabolic adaptations can occur with no concomitant change in oxidative capacity (30, 72, 73). It is possible therefore that the subjects from the TRFA group were trained in comparison to the UTFA group, despite no statistical difference in peak systemic $\dot{V}O_2$, and that the arterial lactate concentrations were lower during infusion in this group due to an enhanced rate of lactate removal.

Although the rate of sodium lactate infusion was increased linearly in the present study, the resulting pattern of increase in the arterial blood lactate concentration was better fit by an exponential function than a linear one. This finding is similar to that of Donovan and Pagliassotti (44), who reported that, during lactate infusion in rats, the relationship between the infusion rate and the arterial blood lactate concentration was best described by a second order regression. The exponential rise in arterial blood lactate concentration in the present study suggests that whole body lactate removal became saturated as the

infusion rate increased, an interpretation which agrees with other studies which have reported lactate removal to be a saturable process (4, 44, 49, 193). However, despite an apparent saturation of whole body lactate removal during lactate infusion, there was no evidence of saturation of net lactate uptake by the inactive forearms in either group. This suggests that the saturation of whole body lactate removal is the result of saturation of lactate removal by some tissue other than skeletal muscle.

In conclusion, the data from the present study indicate that the local muscular adaptations to endurance training which lead to a reduction in net muscle lactate output at a given work load during exercise do not increase the muscles capacity for net lactate removal. This tends to suggest that the reduced net lactate output by trained skeletal muscle during exercise is most likely due to a reduction in net muscle lactate production rather than to an increase in net muscle lactate removal.

## 5.5 FIGURES

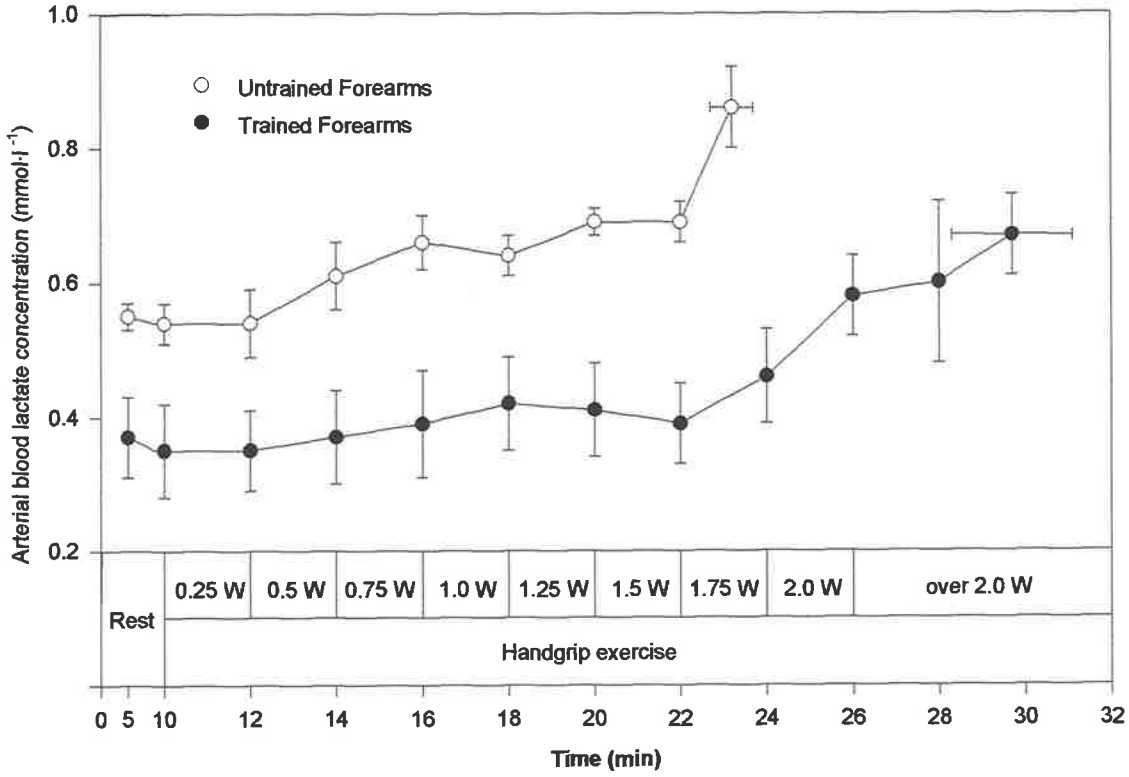


Fig. 5.1. Arterial blood lactate concentrations during rest and incremental handgrip exercise in endurance-trained (n=6) and untrained (n=5) forearms. Values are means ± SEM.

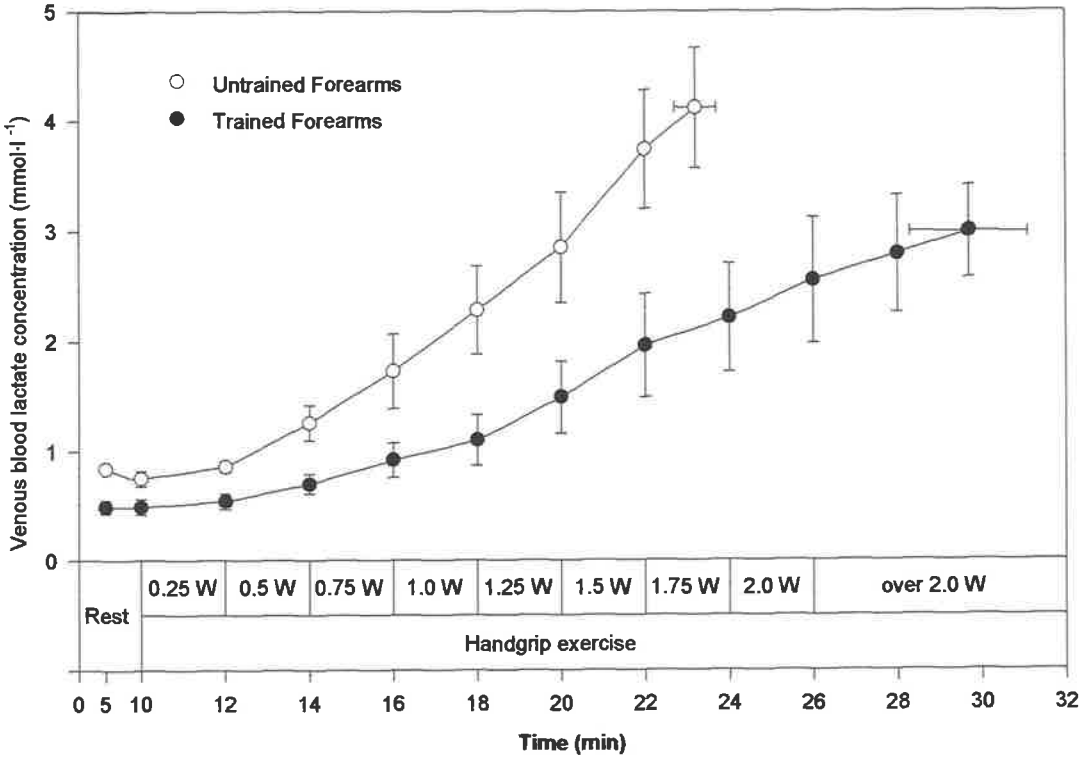


Fig. 5.2. Venous blood lactate concentrations during rest and incremental handgrip exercise in endurance-trained (n=6) and untrained (n=5) forearms. Values are means ± SEM.

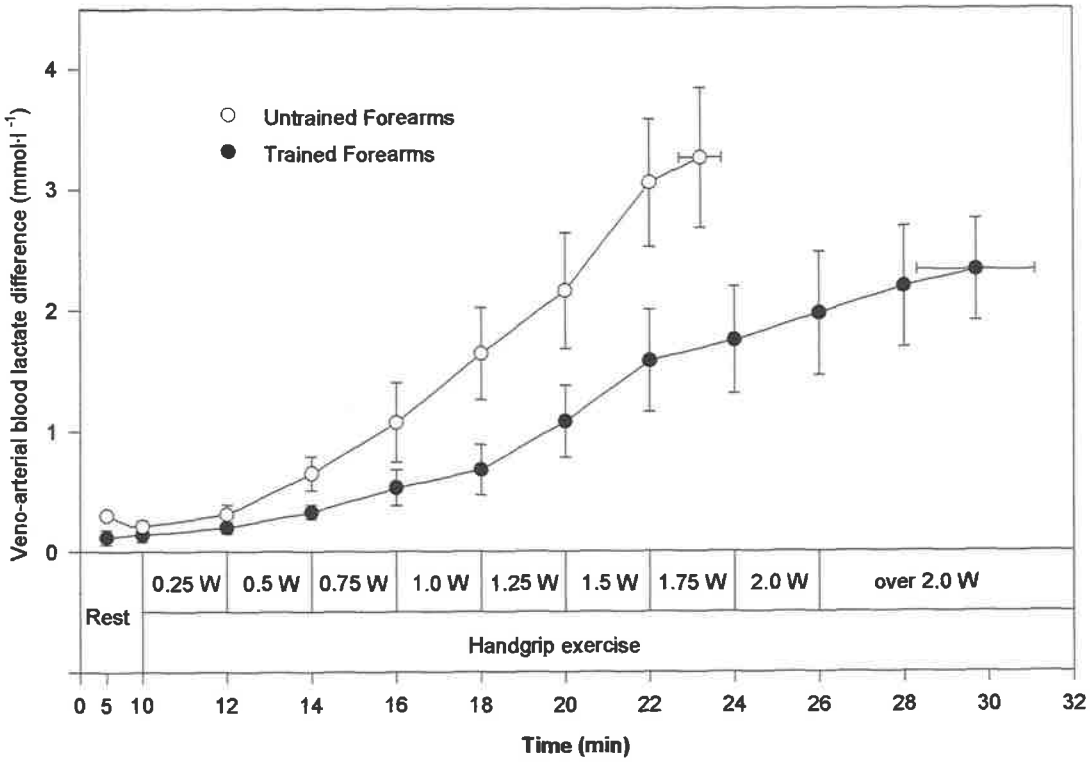


Fig. 5.3. Veno-arterial blood lactate differences during rest and incremental handgrip exercise in endurance-trained (n=6) and untrained (n=5) forearms. Values are means ± SEM.

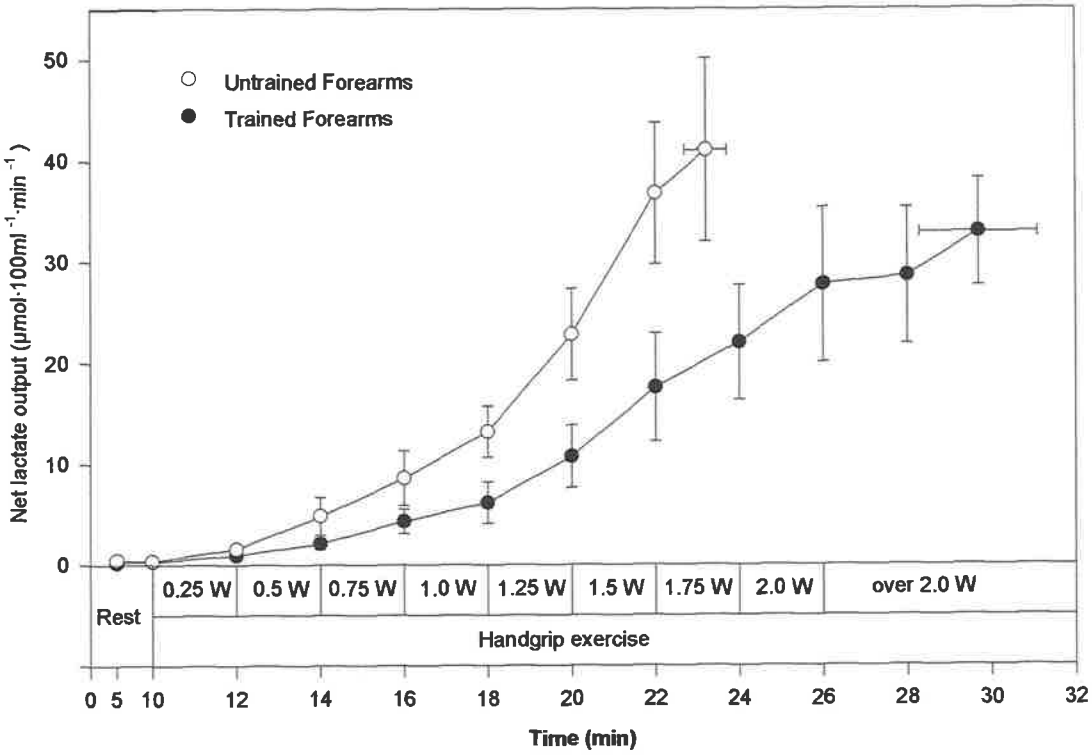


Fig. 5.4. Forearm net lactate output during rest and incremental handgrip exercise in endurance-trained (n=6) and untrained (n=5) forearms. Values are means ± SEM.

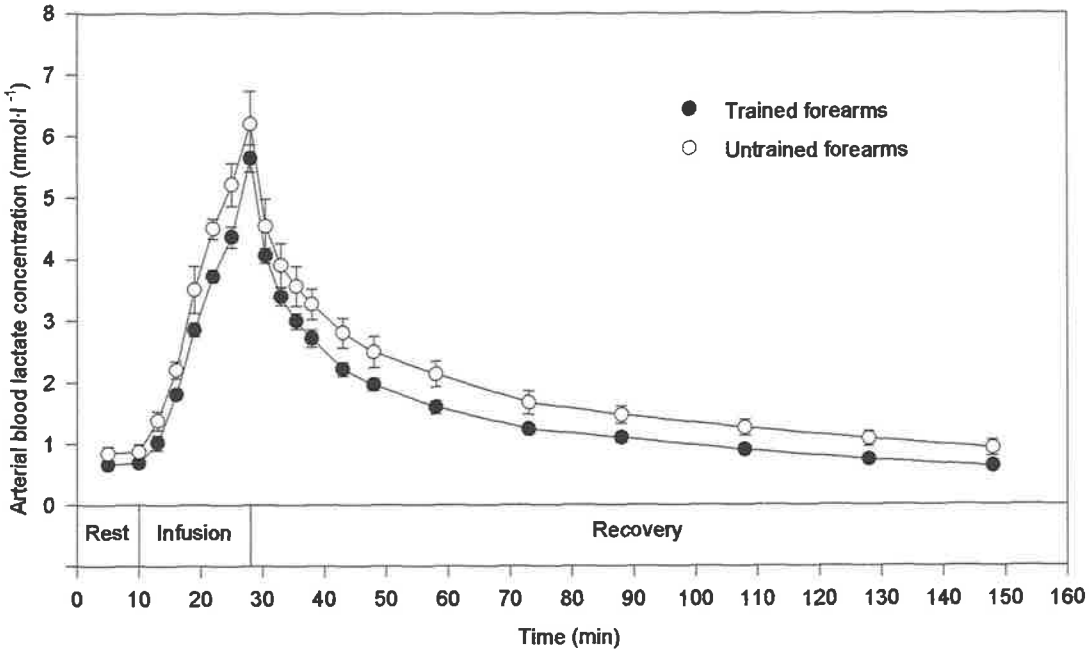


Fig. 5.5. Arterial blood lactate concentrations perfusing endurance-trained (n=6) and untrained (n=5) forearms during rest, incremental sodium lactate infusion and recovery. Values are means ± SEM.

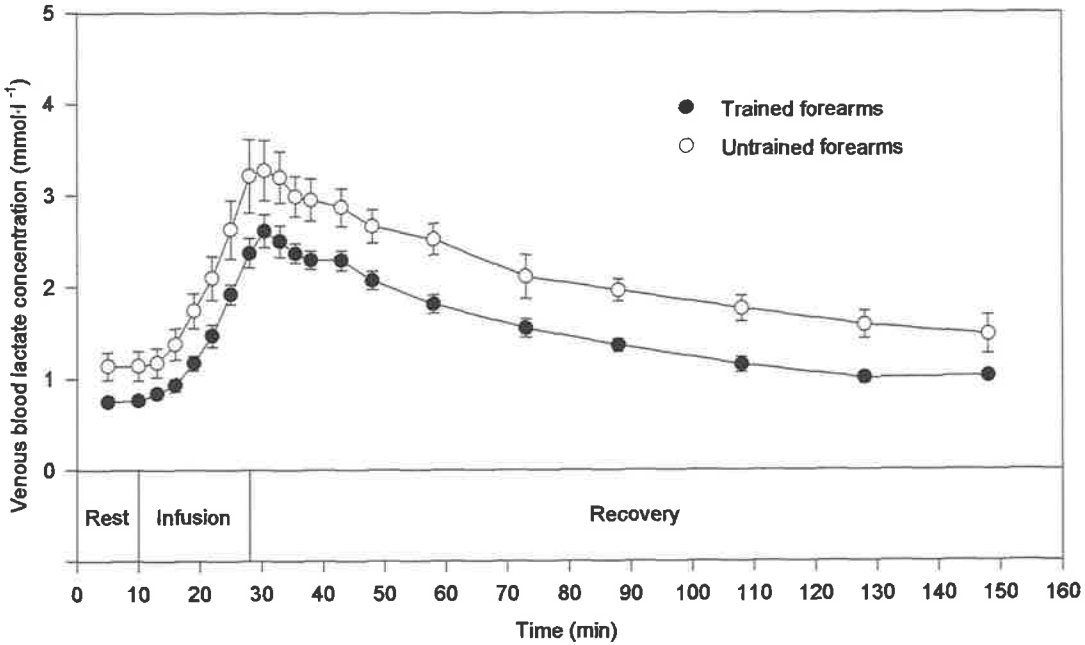


Fig. 5.6. Lactate concentrations in venous blood draining endurance-trained (n=6) and untrained (n=5) forearms during rest, incremental sodium lactate infusion and recovery. Values are means ± SEM.

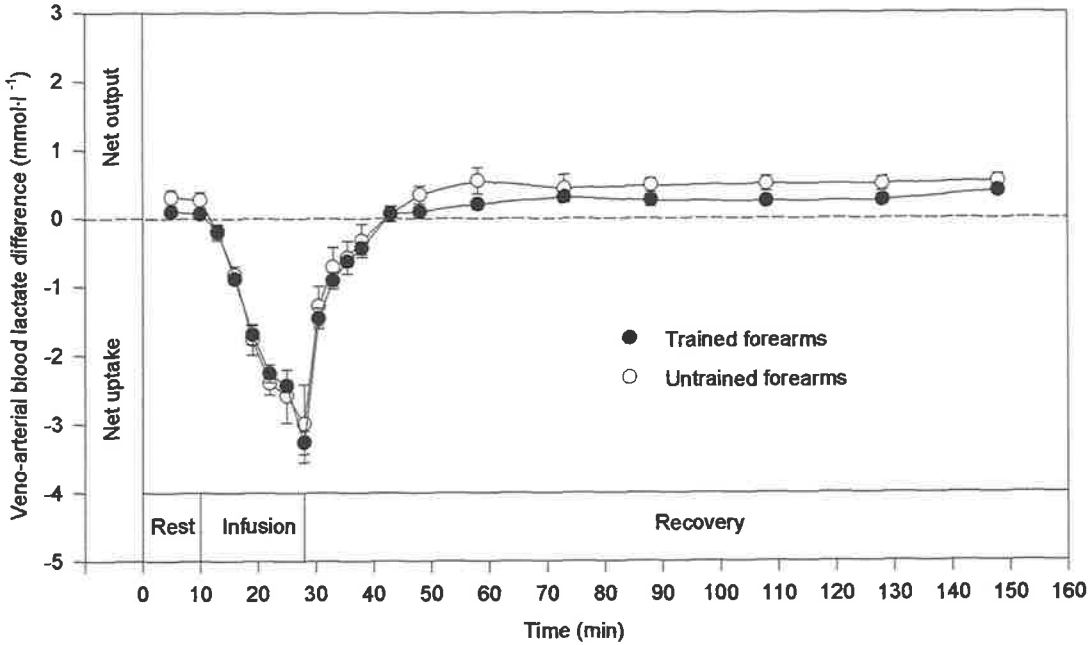


Fig. 5.7. Veno-arterial blood lactate differences across endurance-trained (n=6) and untrained (n=5) forearms during rest, incremental sodium lactate infusion and recovery. Values are means ± SEM.

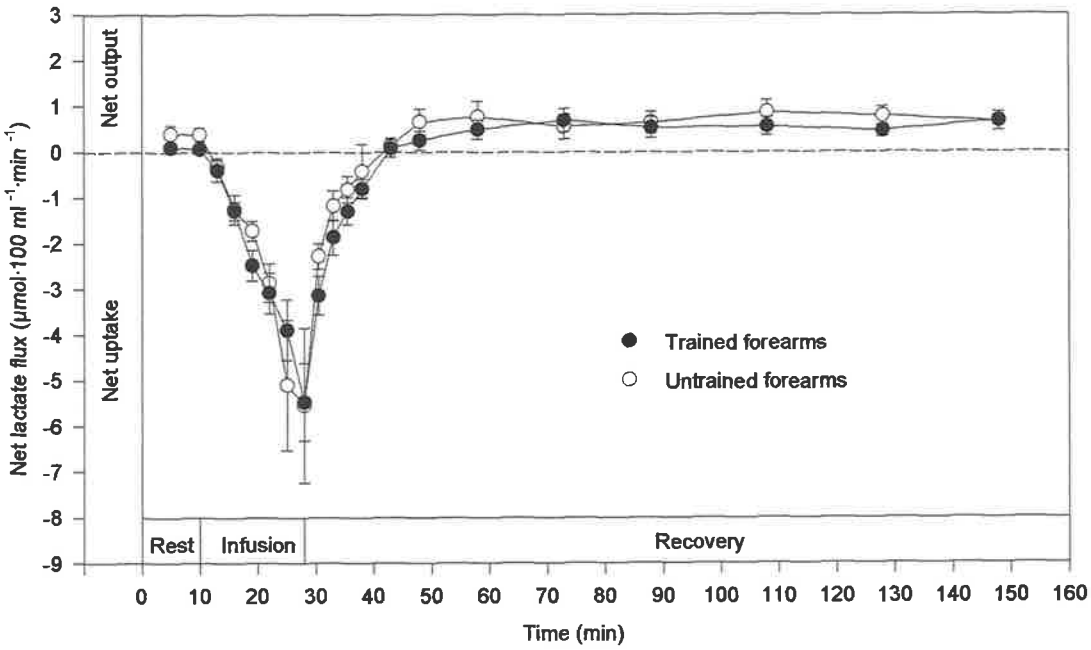


Fig. 5.8. Net lactate flux across endurance-trained (n=6) and untrained (n=5) forearms during rest, incremental sodium lactate infusion and recovery. Values are means ± SEM.

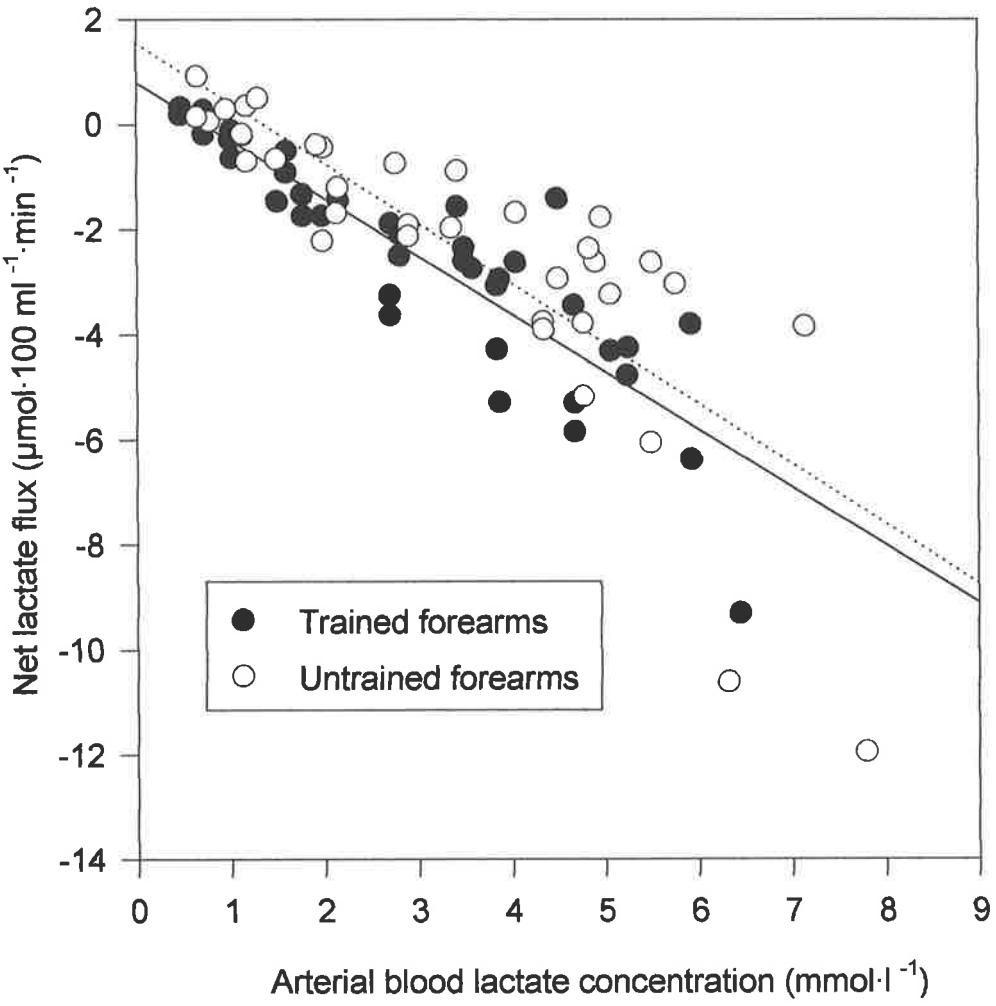


Fig. 5.9. The relationship between arterial blood lactate concentration and forearm net lactate flux during sodium lactate infusion in inactive untrained (n=5) and endurance-trained (n=6) forearms. Values are means ± SEM. Solid line represents endurance-trained forearms regression, dashed line represents untrained forearms regression.

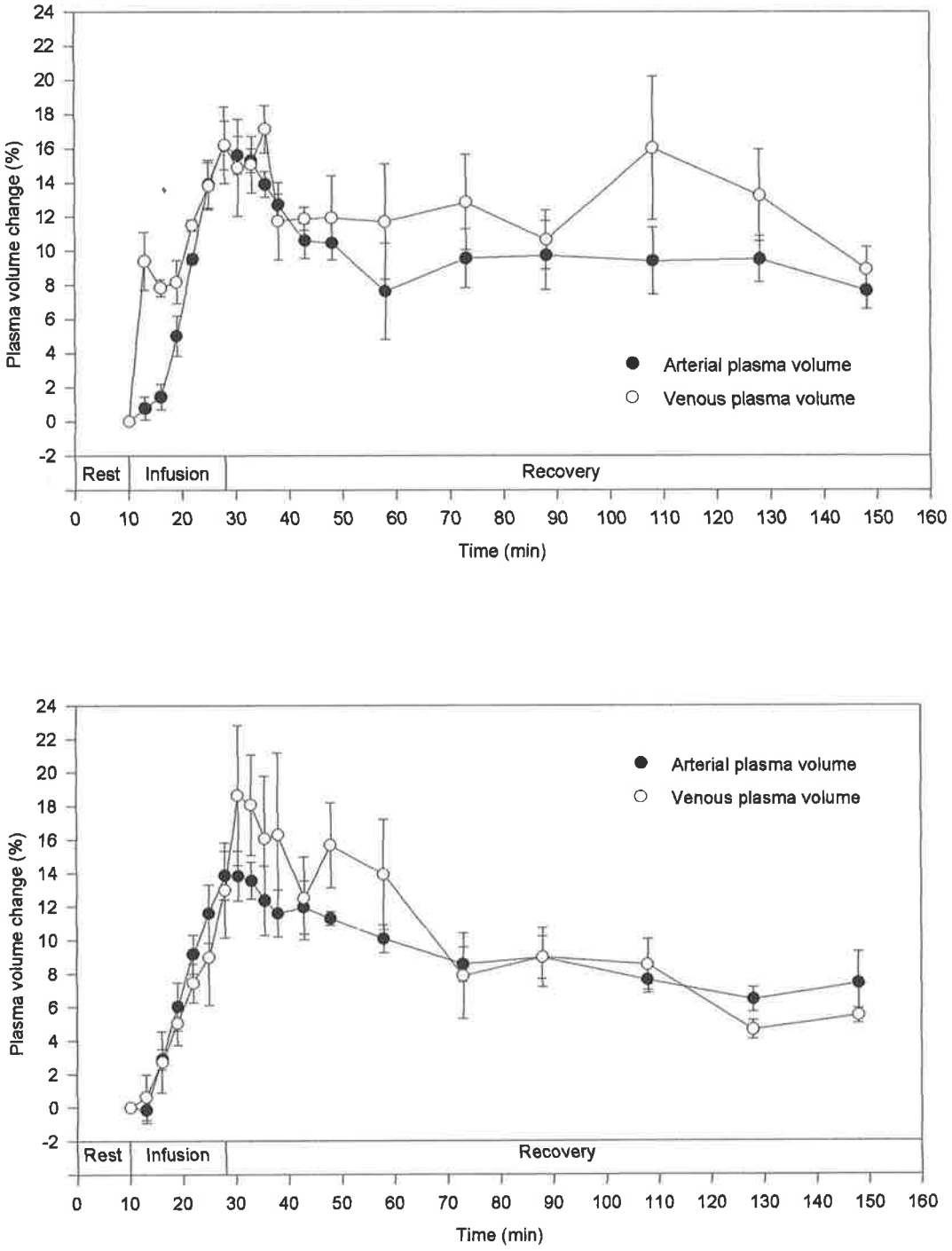


Fig. 5.10. Percentage change in arterial and venous plasma volumes in untrained (upper panel; n=5) and endurance-trained (lower panel; n=6) forearms during incremental sodium lactate infusion and recovery. Values are means ± SEM.

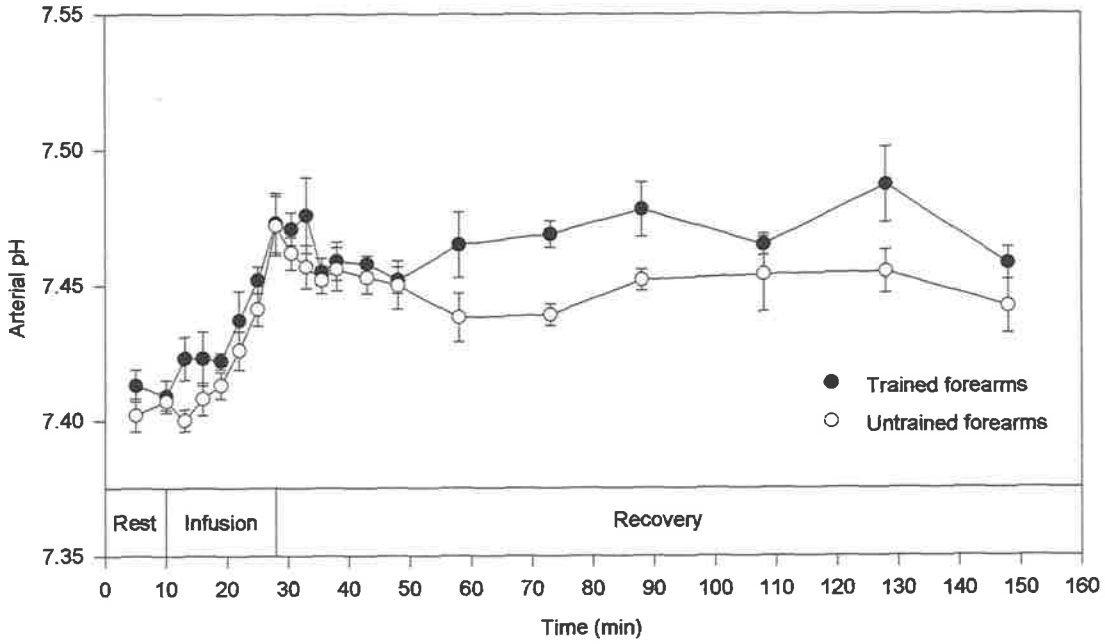


Fig. 5.11. Arterial pH in endurance-trained (n=6) and untrained (n=5) forearms during rest, incremental sodium lactate infusion and recovery. Values are means ± SEM.

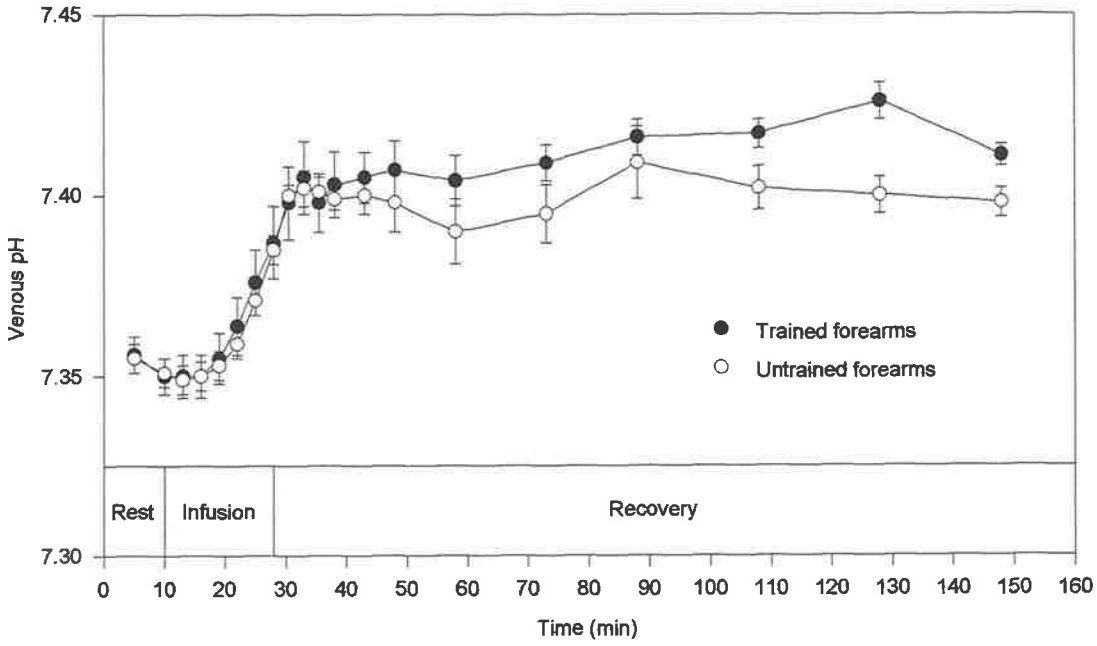


Fig. 5.12. Venous pH in endurance-trained (n=6) and untrained (n=5) forearms during rest, incremental sodium lactate infusion and recovery. Values are means ± SEM.

# CHAPTER 6 - SYSTEMIC TRAINING EFFECTS ON LACTATE REMOVAL BY SKELETAL MUSCLE

## 6.1 INTRODUCTION

Evidence from Chapter 3, as well as from numerous other studies (43-45, 132, 159, 193, 195), suggests that at least part of the reduction in blood lactate concentration at a given work load following training is due to an increase in lactate removal. The tissue responsible for the increased lactate removal is as yet unidentified, however, Donovan and Pagliassotti (44, 45) suggested that localised training adaptations in skeletal muscle might increase the capacity of this tissue for lactate removal. Evidence presented in Chapter 5, as well as the findings of a previous study (28), suggest however that localised training adaptations do not result in an increased capacity for lactate removal in skeletal muscle.

Despite localised training adaptations within skeletal muscle having no apparent effect on lactate removal, there is no information available on the possible effect of systemic adaptations to endurance training on lactate removal by inactive untrained skeletal muscle. When arterial lactate concentrations are elevated above resting levels, inactive skeletal muscle can act as both a passive

sink for lactate, as well as a site of net lactate disposal (3, 28, 31, 163) and, rather than localised adaptations to training increasing the capacity of skeletal muscle to remove lactate from the blood, it is possible that the various cardiovascular, humoral and other systemic adaptations which occur in response to training might promote an environment more conducive to lactate removal by inactive skeletal muscle during exercise.

The purpose of the present study was to compare the uptake and metabolic disposal of lactate by inactive forearm skeletal muscle in endurance-trained and untrained subjects during incremental leg-exercise and recovery, in order to determine whether inactive skeletal muscle contributes to an increased capacity for lactate removal in endurance-trained subjects during exercise.

## 6.2 METHODS

**6.2.1 *General:*** The data in this chapter were collected from six untrained (UT) and six competitive endurance-trained (TR) male cyclists during the whole body exercise experiments reported in Chapter 3. The mean descriptive characteristics of the subjects are reported in Table 6.1.

**Table 6.1.** ***Anthropometric and physiological characteristics of subjects.***

| | UT Subjects (n=6) | TR Subjects (n=6) |
|---|---|---|
| Age (yr) | 25 ± 2.0 | 19.6 ± 0.4† |
| Height (cm) | 174.5 ± 2.1 | 172.8 ± 4.0 |
| Mass (kg) | 68.4 ± 3.3 | 68.7 ± 1.1 |
| Forearm volume (ml) | 1566.7 ± 102.3 | 1590.0 ± 48.4 |
| Forearm circumference (cm) | 25.5 ± 0.9 | 25.7 ± 0.3 |
| Peak systemic oxygen uptake: | | |
| $l \cdot min^{-1}$ | 2.6 ± 0.2 | 3.7 ± 0.2* |
| $ml \cdot kg^{-1} \cdot min^{-1}$ | 38.8 ± 3.2 | 53.2 ± 2.3* |

Values are means ± SEM. * Significantly different from untrained ($P < 0.01$).

† Significantly different from untrained ($P < 0.05$).

The right forearm was studied in all cases. Circumferences and volumes of the right forearm were measured prior to the insertion of a Cathlon IV 20G catheter into the brachial artery of the left arm at the elbow, and an Insyte 18G catheter into the deep muscle branch of the antecubital vein of the right arm. Both of these catheters were used for collection of blood samples. The arterial catheter was also used for monitoring arterial blood pressure.

Approximately 30 min after the catheters had been inserted a 10 min rest period was commenced during which basal measurements of FBF, HR, MAP and pulsatile blood pressure were recorded. Subjects then performed incremental supine cycling exercise on a Jaquet Supine Cycle ergometer. The pedal rate was set at 60 rpm with an initial work load of 100 W. The work load was increased by 25 W every 10 min until volitional exhaustion, and was followed by a two hour recovery period. Systemic oxygen uptake was measured only during the initial 10 min rest period and exercise, whereas FBF, HR, MAP and pulsatile blood pressure were measured throughout the protocol.

**6.2.2 *Blood sampling:*** Simultaneous samples of arterial and venous blood (4.5 ml each) were obtained at 5 and 10 min of the initial 10 min rest period, every 2.5 min during exercise, and at 2.5, 5, 7.5 and 10 min of recovery and every 10 min thereafter up to 120 min. These blood samples were analysed for whole blood lactate concentrations, blood gases, $\%SO_2$ and [tHb]. Due to technical difficulties, blood gases, $\%S_vO_2$ and [tHb] were not obtained for the blood samples from one UT subject.

## 6.3 RESULTS

**6.3.1 *Performance characteristics:*** The TR subjects were able to reach significantly higher peak work loads during exercise (UT, 179.2 ± 10.0 W; TR,

241.7 ± 8.3 W; P<0.001), and performed more than twice as much total work as the UT subjects when expressed in either absolute terms (UT, 304.4 ± 41.6 kJ; TR, 638.1 ± 45.1 kJ; P<0.001) or relative to body mass (UT, 4.5 ± 0.6 $kJ \cdot kg^{-1}$; TR, 9.3 ± 0.6 $kJ \cdot kg^{-1}$; P<0.001).

**6.3.2 *Systemic Oxygen Uptake:*** There was no difference in systemic oxygen uptake ($\dot{V}O_2$) between the two groups prior to exercise (UT, 3.9 ± 0.2 $ml \cdot kg^{-1} \cdot min^{-1}$; TR, 4.6 ± 0.5 $ml \cdot kg^{-1} \cdot min^{-1}$), or at comparable work loads during exercise. However, because the TR subjects were able to reach higher work loads prior to exhaustion they achieved higher absolute and relative values for peak systemic $\dot{V}O_2$ than the UT subjects (Table 6.1).

**6.3.3 *Systemic Respiratory Exchange Ratio:*** The systemic RER was similar in both groups prior to exercise (UT, 0.83 ± 0.05; TR, 0.88 ± 0.04). During exercise, the systemic RER increased progressively in both groups, but was significantly higher in the UT group, not only at comparable work loads, but also at the end of exercise (UT, 1.12 ± 0.03; TR, 0.92 ± 0.03).

**6.3.4 *Heart Rate:*** There was no difference in HR between the two groups prior to exercise (UT, 70 ± 8 $beats \cdot min^{-1}$; TR 65 ± 5 $beats \cdot min^{-1}$). During exercise, HR increased in both groups, but was significantly lower in the TR subjects at comparable work loads. Nevertheless, similar peak heart rates were reached in

both groups at the end of exercise (UT, 185 ± 4 beats·min$^{-1}$; TR 184 ± 5 beats·min$^{-1}$). Following the cessation of exercise, HR decreased at a similar rate and had returned to pre-exercise values in both groups by the end of recovery.

**6.3.5 *Mean Arterial Blood Pressure:*** MAP during rest, exercise and recovery are shown in Fig. 6.1. There was no difference in MAP between the two groups prior to exercise (UT 93.7 ± 2.1 mmHg; TR 92.0 ± 4.3 mmHg). During exercise MAP increased rapidly in the UT group, resaching peak values of 123.3 ± 5.9 mmHg at the end of exercise. However, MAP did not increase in the TR group during exercise ($P>0.05$), resulting in MAP being significantly higher in the UT group at comparable work loads. During recovery MAP decreased rapidly in both groups, initially reaching values lower than those recorded prior to exercise, before gradually returning to pre-exercise values in both groups by the end of the recovery period.

**6.3.6 *Forearm Blood Flow and percentage venous oxyhaemoglobin saturation:*** FBF and percentage venous oxyhaemoglobin saturation ($\%S_vO_2$) during rest, exercise and recovery are shown in Fig. 6.2 and Fig. 6.3 respectively. There was no difference in FBF (UT, 3.5 ± 0.5 ml·100ml$^{-1}$·min$^{-1}$; TR, 2.8 ± 0.5 ml·100ml$^{-1}$·min$^{-1}$) or $\%S_vO_2$ (UT, 72.9 ± 2.5%; TR, 63.0 ± 7.8%) between the two groups at rest. During exercise FBF increased in both groups, with no difference between the two groups at comparable work loads. However, by the end of exercise FBF in the TR group had reached values more than

two-fold higher than in the UT group (UT, 4.8 ± 0.7 ml·100ml$^{-1}$·min$^{-1}$; TR, 10.8 ± 2.5 ml·100ml$^{-1}$·min$^{-1}$). FBF during exercise was not significantly correlated with MAP in either group (UT, r=0.41; TR, r=0.65; P>0.05). However, FBF was highly correlated with forearm vascular conductance (UT, r=0.97; TR, r=1.00; P<0.05), with the correlation coefficient being significantly higher in the TR group (P<0.05). $\%S_vO_2$ decreased initially in both groups with the onset of exercise, before returning to pre-exercise values in the UT group (73.2 ± 8.2%) and increasing to values significantly higher than those at rest in the forearms of the TR subjects (82.8 ± 7.8%) by the end of exercise. During recovery FBF and $\%S_vO_2$ exhibited transient increases in the forearms of the UT subjects, before returning to pre-exercise values by the end of recovery. $\%S_vO_2$ also exhibited an initial increase in the forearms of the TR subjects during recovery, before declining toward pre-exercise values, but FBF exhibited a rapid and progressive decline throughout the recovery period. By the end of recovery, both FBF and $\%S_vO_2$ had also returned to pre-exercise values in the forearms of the TR subjects.

**6.3.7 *Forearm Respiratory Variables:*** There was no difference in $FA\dot{V}O_2$ between the two groups prior to exercise (UT, 7.5 ± 0.5 µmol·100ml$^{-1}$·min$^{-1}$; TR, 7.3 ± 0.5 µmol·100ml$^{-1}$·min$^{-1}$) and these values did not change significantly in either group during exercise or recovery.

The FARER was also similar in both groups prior to exercise (UT, 0.98 ± 0.11; TR, 0.93 ± 0.09). During exercise, the FARER increased in both groups, but was significantly higher in the forearms of the UT subjects at comparable submaximal work loads. However, by the end of exercise similar values for FARER had been reached in both groups (UT, 2.25 ± 0.38; TR, 2.28 ± 0.68). During recovery the FARER declined at a similar rate in both groups, and had returned to pre-exercise values by the end of recovery.

**6.3.8 *Blood lactate:*** The arterial and venous blood lactate concentrations during rest, exercise and recovery in the UT and TR subjects are shown in Fig. 6.4 and Fig. 6.5 respectively. Prior to the commencement of exercise there were no differences in arterial (UT, 0.68 ± 0.10 $mmol \cdot l^{-1}$; TR, 0.77 ± 0.10 $mmol \cdot l^{-1}$) or venous (UT, 0.71 ± 0.15 $mmol \cdot l^{-1}$; TR, 0.89 ± 0.13 $mmol \cdot l^{-1}$) blood lactate concentrations between the two groups. The arterial and venous blood lactate concentrations increased in both groups during exercise, but were significantly lower in the TR group at comparable submaximal work loads. However, by the end of exercise, there was no difference in arterial (UT, 7.72 ± 0.86 $mmol \cdot l^{-1}$; TR, 5.08 ± 1.72 $mmol \cdot l^{-1}$) or venous (UT, 5.36 ± 0.59 $mmol \cdot l^{-1}$; TR, 4.0 ± 1.41 $mmol \cdot l^{-1}$) blood lactate concentrations between the two groups. During recovery the arterial and the venous blood lactate concentrations declined rapidly in both groups, and had returned to pre-exercise values by the end of recovery.

Veno-arterial blood lactate differences during rest, exercise and recovery are shown in Fig. 6.6 There was a small, positive, v-a lactate difference in both groups of forearms prior to the commencement of exercise (UT, 0.03 ± 0.08 $mmol \cdot l^{-1}$; TR, 0.12 ± 0.10 $mmol \cdot l^{-1}$; $P>0.05$). With the onset of exercise the v-a lactate difference quickly became negative in the UT group, indicating net lactate uptake, but did not change from pre-exercise values in the forearms of the TR group. During recovery, the v-a lactate difference remained unchanged in the forearms of the TR group, whilst returning to pre-exercise values in the forearms of UT group.

Net forearm lactate flux during rest, exercise and recovery is shown in Fig. 6.7. There was a tendency toward net lactate output by the forearms of both groups prior to exercise, but in the UT group this net output did not reach statistical significance (UT, 0.2 ± 0.2 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$; TR 0.4 ± 0.2 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$). With the onset of supine cycling exercise, the forearms of the UT subjects quickly began to take up lactate on a net basis, reaching a maximal rate of 10.6 ± 2.6 $\mu mol \cdot 100ml^{-1} \cdot min^{-1}$ by the end of exercise. This was in contrast to the forearms of the TR group, which did not demonstrate any significant net lactate uptake during either exercise or recovery. During recovery the forearms of the UT subjects released less lactate into the circulation (90.3 ± 35.4 $\mu mol \cdot 100ml^{-1}$) than they had taken up during exercise (248.0 ± 45.5 $\mu mol \cdot 100ml^{-1}$), representing a net metabolic disposal of 157.8 ± 49.7 $\mu mol \cdot 100ml^{-1}$ of lactate, or 16.2 ± 6.3% of the total arterial lactate load delivered

to this group of forearms during the exercise and recovery periods. In comparison, the forearms of the TR subjects demonstrated a net lactate production of 73.0 ± 30.1 μmol·100ml$^{-1}$ for the same period.

The rate of net forearm lactate flux was negatively correlated with the arterial blood lactate concentration in both groups of forearms during exercise (UT, r=-0.66; TR, r=-0.46; P<0.0001) (Fig. 6.8). There was no statistical difference in this relationship between the two groups, so the data were pooled, yielding an overall correlation of r= -0.57 (P<0.0001) and an overall regression equation of y= -1.87x + 2.55.

## 6.4 DISCUSSION

If the lower blood lactate concentrations during exercise in endurance-trained subjects are due to an increased rate of lactate removal, evidence from the present study suggests that inactive skeletal muscle is not the tissue responsible. The TR subjects demonstrated significant adaptations to training in comparison to the UT subjects, achieving higher absolute and relative values for peak systemic $\dot{V}O_2$ (Table 6.1), lower arterial and venous blood lactate concentrations, and lower HR, MAP and systemic RER values at comparable submaximal work loads during exercise. However, despite the lower arterial and venous blood lactate concentrations the inactive forearms of the TR subjects

were a site of net lactate production, whereas the inactive forearms of the UT subjects took up and disposed of a significant quantity of lactate, indicating that inactive skeletal muscle does not contribute to an increased rate of lactate removal in endurance-trained subjects.

Net uptake and metabolic disposal of lactate was only apparent in the forearms of the UT subjects, the forearms of the TR subjects neither took up, nor disposed of, any significant quantity of lactate during exercise or recovery. Given that the rate of forearm net lactate flux was negatively correlated with the arterial lactate concentration in both groups of forearms during exercise (UT, $r=-0.66$; TR, $r=-0.46$; $P<0.0001$), such that net forearm lactate uptake increased with increases in the arterial lactate concentration, the difference in lactate metabolism between the two groups of forearms could be explained on the basis of the lower arterial blood lactate concentration in the TR subjects during exercise. Other studies have also shown that the rate of lactate uptake by inactive skeletal muscle is related to the arterial lactate concentration (28, 71), but the present study was the first to demonstrate that there is no difference in the rate of lactate uptake by inactive skeletal muscle at a given arterial lactate concentration between endurance-trained and untrained subjects (Fig. 6.8). This latter finding suggests that the numerous systemic adaptations which occur in response to endurance exercise training do not provide an internal milieu which favours an increased rate of lactate removal by inactive skeletal muscle. Instead, it appears that the effects of endurance training on lactate metabolism by inactive skeletal

muscle are secondary to the adaptations in other tissues which serve to reduce the arterial blood lactate concentration at a given work load.

Although there was no difference in the rate of net forearm lactate uptake at a given arterial blood lactate concentration between the two groups, analysis of the regression equations describing this relationship indicated that the arterial lactate concentration need only increase by 0.7-0.8 $mmol.l^{-1}$ above resting levels for inactive skeletal muscle to begin taking up lactate from the blood. That the magnitude of the increase in arterial lactate concentration required to stimulate net forearm lactate uptake is so small demonstrates the sensitivity of inactive skeletal muscle as a buffer to increases in blood lactate during exercise. Buffering of increases in blood lactate by inactive skeletal muscle could play an important role in maintaining contractile function of the active muscles during exercise by helping to maintain a favourable muscle-blood gradient for the release of lactate from the active muscles. Since lactate accumulation inhibits contractile function in working muscle (87), an increased movement of lactate out of the active skeletal muscles would help to reduce intramuscular lactate accumulation and delay contractile fatigue, leading to an improved capacity to sustain exercise. That inactive skeletal muscle can provide such a sensitive buffer, combined with the fact that this tissue comprises approximately 45% of body mass in non-obese humans (163) is indicative of the importance of inactive skeletal muscle as a regulator of blood lactate concentrations during exercise.

Although the rate of net forearm lactate flux was negatively correlated with the arterial lactate concentration during exercise (r=-0.57) in the present study, this relationship was not as strong as when lactate infusion was used to elevate the arterial lactate concentration in Chapter 5 (r=-0.95). The difference in the strength of this relationship between the two studies was most likely related to differences in cutaneous blood flow. In the present study FBF increased in both groups during exercise, whereas in Chapter 5, the lactate infusions used to elevate the arterial blood lactate concentration did not result in any change in FBF. Blood flow through a tissue can increase as a result of two processes, an increase in perfusion pressure, or an increase in vascular conductance. In the present study the finding of a high correlation between FBF and vascular conductance in both groups during exercise (UT, r=0.97; TR, r=1.00; $P<0.05$), but no significant correlation between FBF and MAP (UT, r=0.41; TR, r=0.65; $P>0.05$), suggests that the increase in FBF during exercise in the present study was due to an increase in vascular conductance. The method of venous occlusion plethysmography used to measure FBF in the studies outlined in this thesis measures total FBF and it is not possible to differentiate between the relative contributions of cutaneous and muscle blood flows to total FBF. However, given that the leg exercise used to elevate the arterial blood lactate concentration in the present study would have generated a significant metabolic heat load, it is likely that the increased vascular conductance observed in the forearms of both groups of subjects during exercise resulted from increases in cutaneous blood flow to dissipate the thermal load. Such an increase in

cutaneous blood flow would have led to a weakening of the relationship between the arterial lactate concentration and net lactate flux in the present study compared to when lactate infusions were used in Chapter 5, since an increase in cutaneous blood flow would result in much of the arterial blood bypassing the inactive forearm skeletal muscles, thereby reducing the opportunity for the muscles to take up lactate from the blood.

The forearms of the UT subjects took up more lactate during exercise ($248.0 \pm 45.5$ $\mu mol \cdot 100ml^{-1}$) than was released to the circulation during recovery ($90.3 \pm 35.4$ $\mu mol \cdot 100ml^{-1}$), resulting in a calculated total net lactate disposal of $157.8 \pm 49.7$ $\mu mol \cdot 100ml^{-1}$. The forearms of the TR subjects on the other hand, did not take up any significant quantity of lactate during exercise, and demonstrated a total net lactate production of $73.0 \pm 30.1$ $\mu mol \cdot 100ml^{-1}$. The differences in forearm lactate metabolism between the two groups were most likely related to the differences in arterial lactate concentrations. It has been suggested that lactate disposal by inactive skeletal muscle is dependent on the concentration of lactate in the arterial blood perfusing the muscle (28, 71). In the present study, the arterial lactate concentration increased rapidly with the onset of exercise in the UT subjects, but increased much more slowly in the TR group. If net lactate disposal by inactive skeletal muscle is dependent on the arterial blood lactate concentration, the significantly higher arterial blood lactate concentrations in the UT subjects during exercise could account for the fact that

the forearms of those subjects disposed of a significant quantity of blood lactate, whereas the forearms of the TR subjects did not.

Some studies have reported that the $\dot{V}O_2$ of inactive skeletal muscle increases in association with net lactate disposal during both exercise (3) and lactate infusion (4), and have suggested that $\dot{V}O_2$ increases as a result of lactate oxidation. However, in the present study, and others which have also examined lactate removal by inactive skeletal muscle (28, 32), net lactate disposal was found to occur despite no concurrent increase in $\dot{V}O_2$. That net lactate disposal can occur independently of changes in $\dot{V}O_2$ suggests that in those studies which have reported an increase in $\dot{V}O_2$ associated with net lactate disposal (3, 4) some other event temporally related to lactate disposal is responsible for the increase in $\dot{V}O_2$. In any case, an increase in $\dot{V}O_2$ of an inactive skeletal muscle need not be a prerequisite for net lactate disposal, a shift in substrate from fat to lactate could result in net lactate disposal with little or no change in $\dot{V}O_2$. Evidence that a shift in substrate utilisation by the forearms of the UT group occurred in the present study comes from the observation that the forearms of the UT group demonstrated a net disposal of lactate with no change in $FA\dot{V}O_2$, indicating that lactate was disposed of in place of, rather than in addition to, normal substrate usage. Also, prior to the commencement of exercise the mean RER of the inactive forearms of the UT group was 0.87, but this increased to greater than

unity with the onset of exercise, suggesting that the inactive forearms changed their pattern of substrate utilisation by reducing fat usage and increasing carbohydrate utilisation, presumably due to an increased oxidation of lactate taken up from the blood. Furthermore, it was calculated that if lactate was the sole substrate for the inactive forearms, enough oxygen was available to account for more than twice (235.15 ± 83.91%) the quantity of lactate disposed of.

The principal finding of the present study was that rather than contributing to an increased rate of lactate removal in endurance-trained subjects, the adaptations in lactate metabolism by inactive skeletal muscle that occur in response to endurance exercise training are secondary to the adaptations in other tissues which reduce the arterial blood lactate concentration at a given work load.

## 6.5 FIGURES

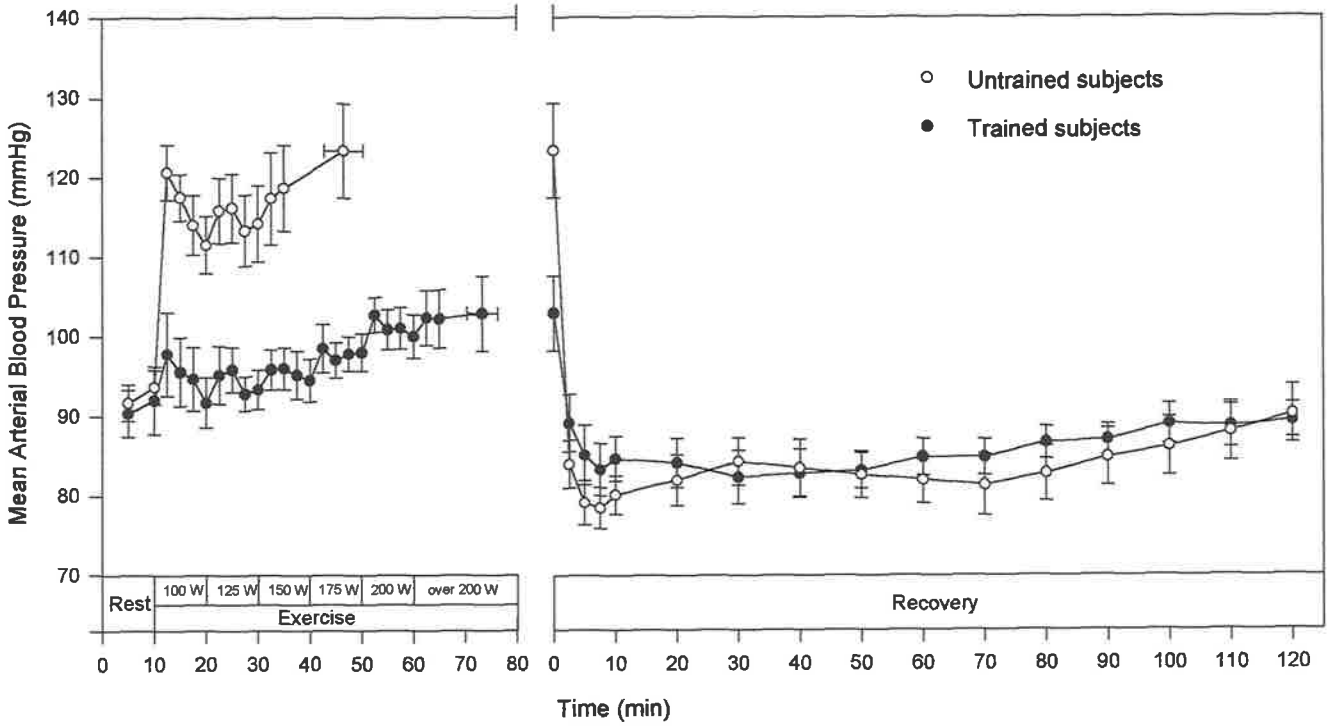


Fig. 6.1 Mean arterial blood pressure during rest, incremental supine cycling exercise and passive recovery in untrained (n=6) and endurance-trained (n=6) subjects. Values are means ± SEM.

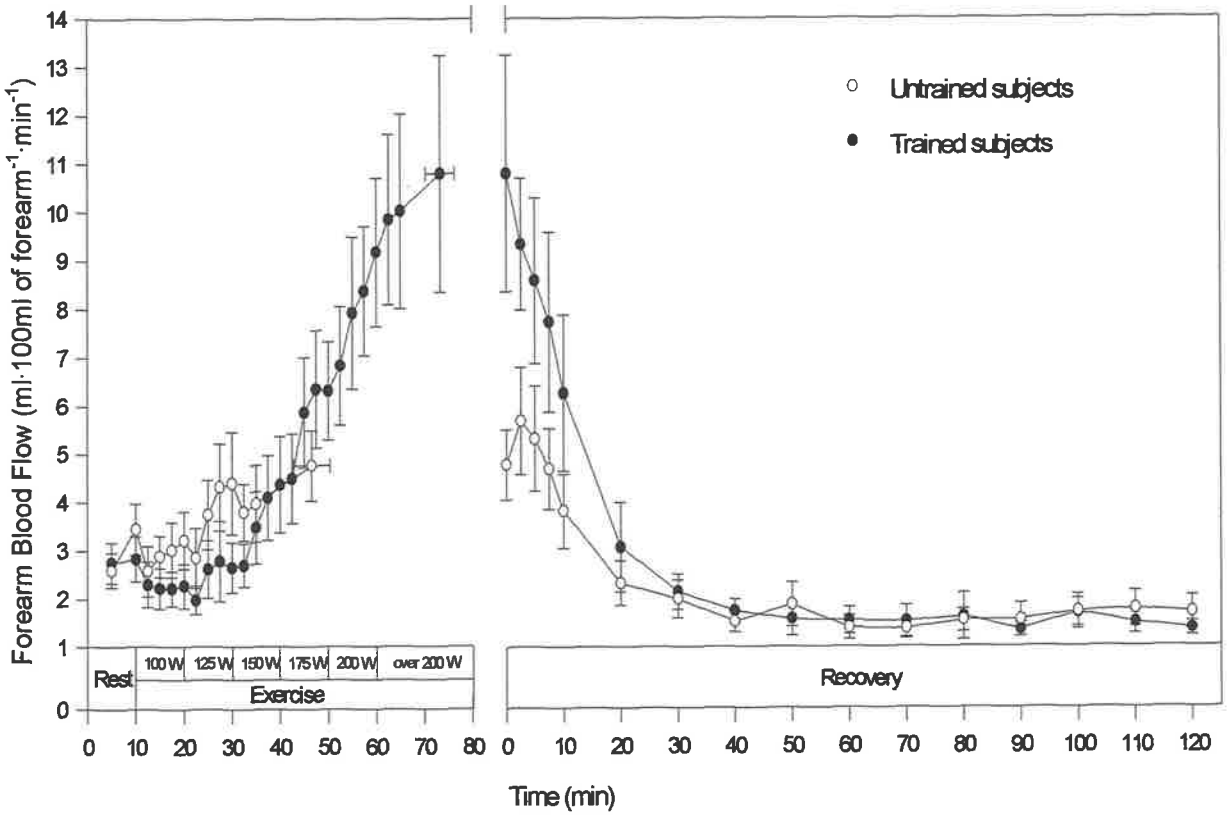


Fig. 6.2 Forearm blood flow during rest, incremental supine cycling exercise and passive recovery in untrained (n=6) and endurance-trained (n=6) subjects. Values are means ± SEM.

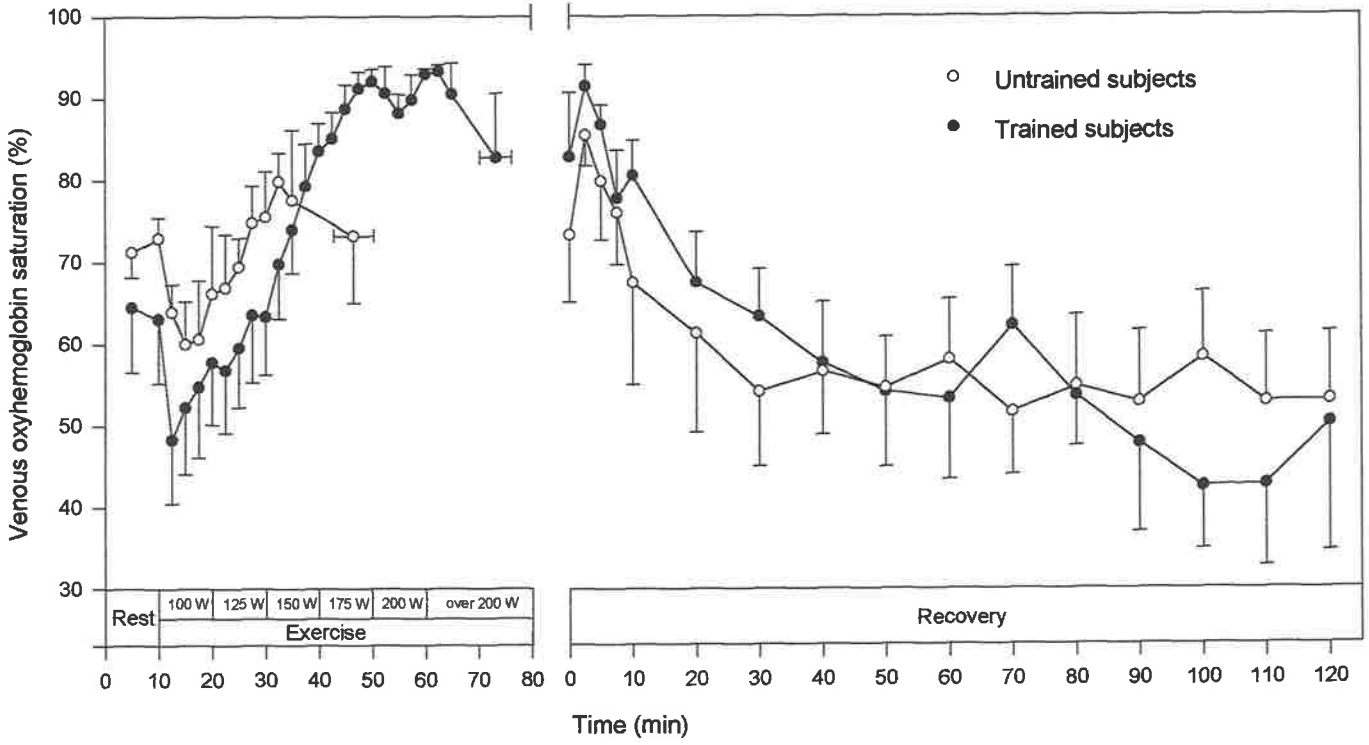


Fig. 6.3 Percentage venous oxyhaemoglobin saturation during rest, incremental supine cycling exercise and passive recovery in untrained (n=5) and endurance-trained (n=6) subjects. Values are means ± SEM.

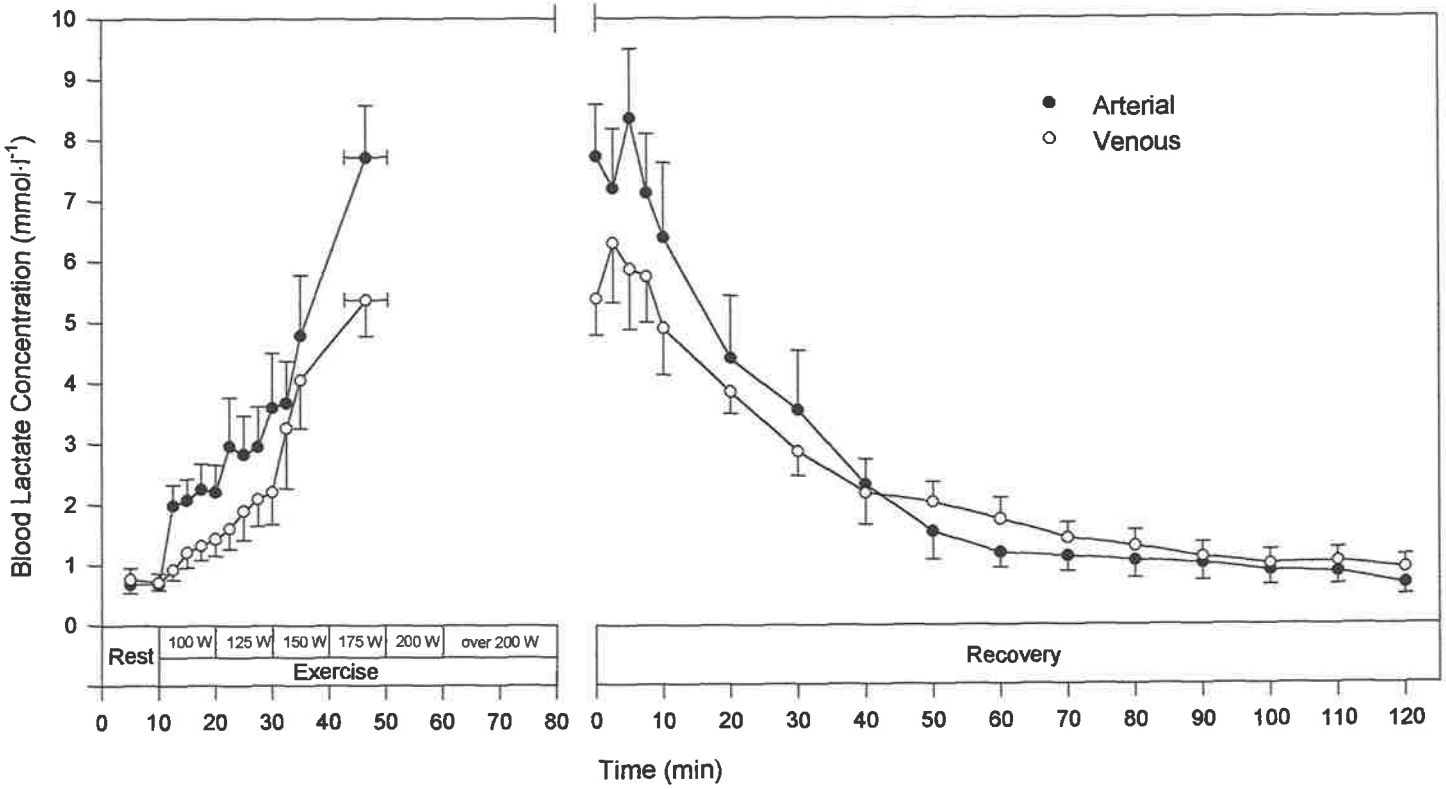


Fig. 6.4. Arterial and venous blood lactate concentrations during rest, incremental supine cycling exercise and passive recovery in untrained (n=6) subjects. Values are means ± SEM.

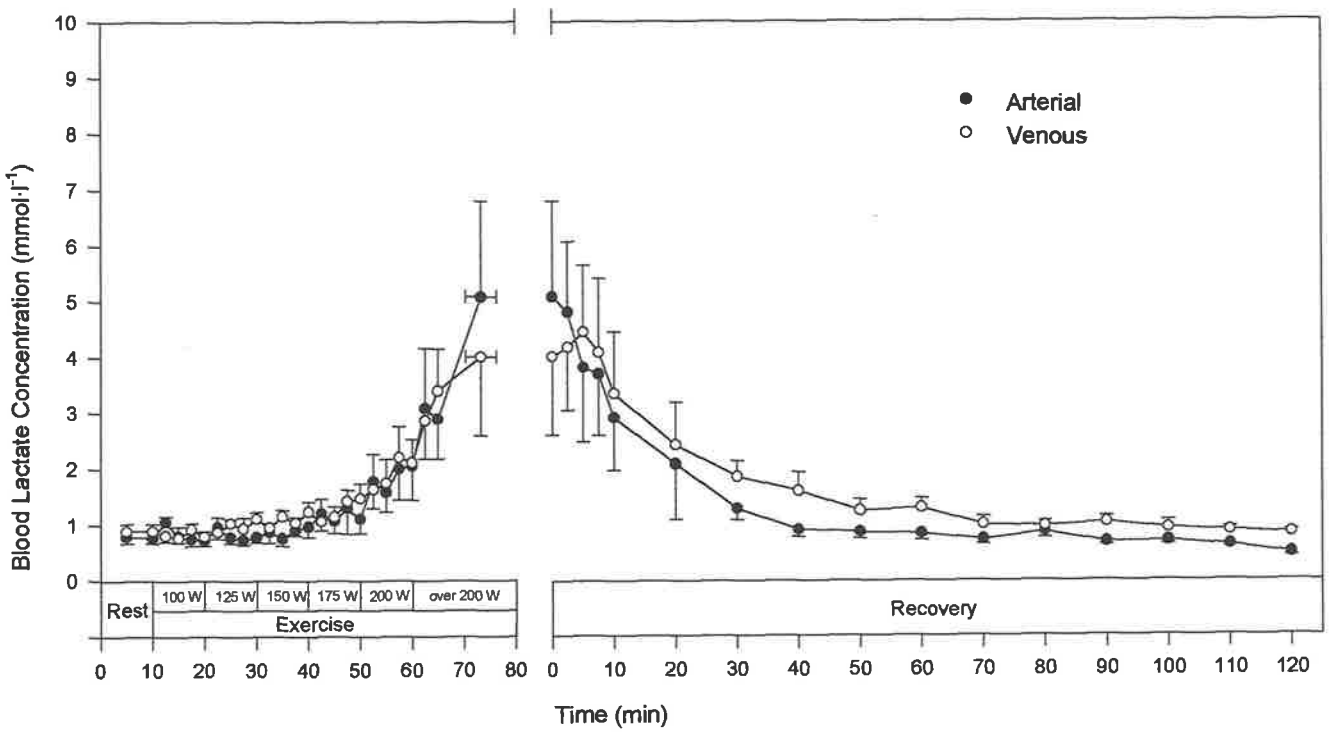


Fig. 6.5. Arterial and venous blood lactate concentrations during rest, incremental supine cycling exercise and passive recovery in endurance-trained (n=6) subjects. Values are means ± SEM.

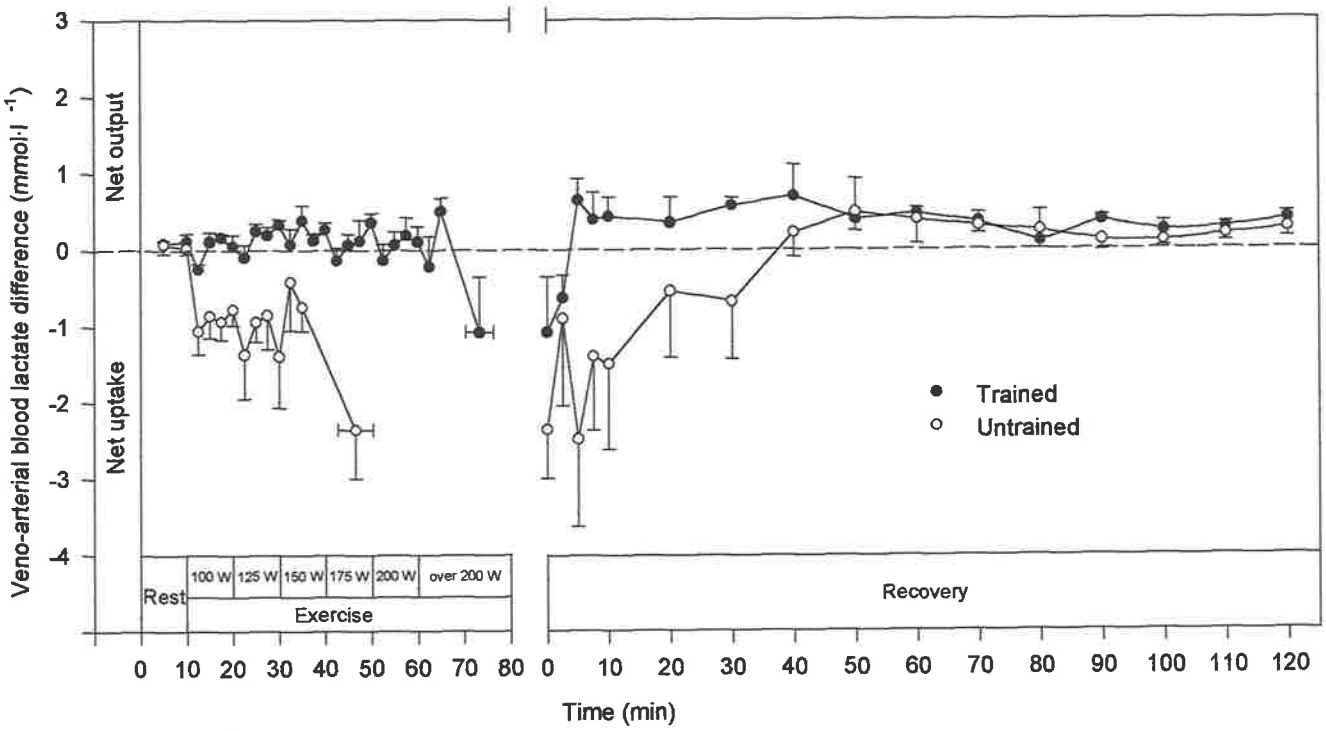


Fig. 6.6. Veno-arterial blood lactate difference during rest, incremental supine cycling exercise and passive recovery in untrained (n=6) and endurance-trained (n=6) subjects. Values are means ± SEM.

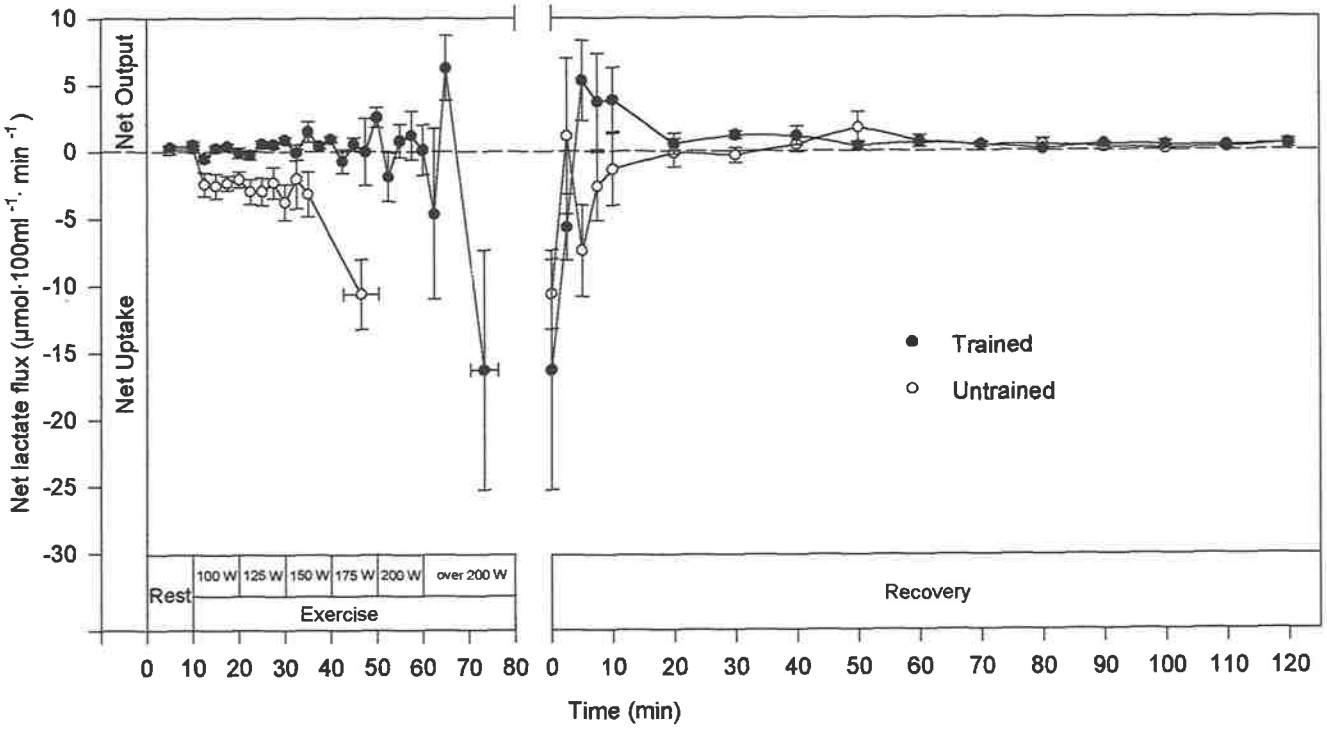


Fig. 6.7. Net lactate flux during rest, incremental supine cycling exercise and passive recovery in untrained (n=6) and endurance-trained (n=6) subjects. Values are means ± SEM.

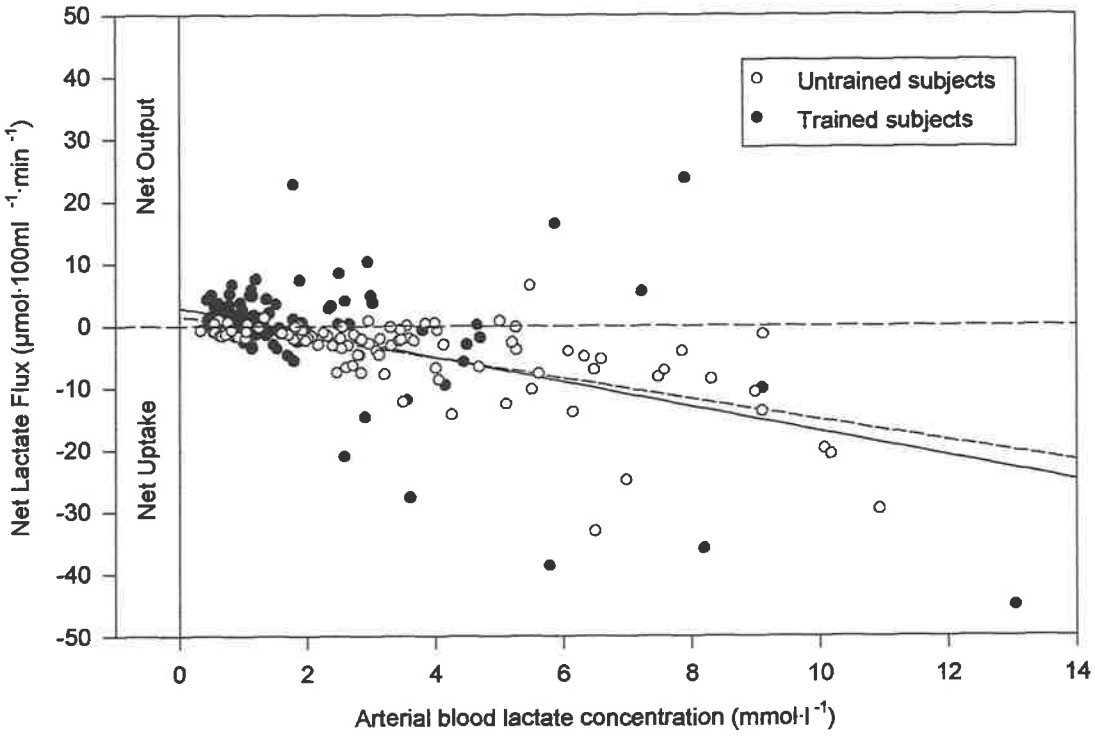


Fig. 6.8. Net lactate flux as a function of the arterial blood lactate concentration during incremental supine cycling exercise in untrained (n=6) and endurance-trained (n=6) subjects. Untrained subjects regression - broken line. Trained subjects regression - solid line.

# CHAPTER 7 - SUMMARY AND CONCLUSIONS

The work described in this thesis focussed primarily on determining whether the lower blood lactate concentrations in endurance-trained individuals at a given submaximal work load are attributable to a reduced rate of lactate production or an increased rate of lactate removal, with a particular focus on the role played by skeletal muscle.

Numerous isotopic tracer studies have indicated that endurance exercise training enhances lactate removal (43-45, 132, 159, 193, 195), and that the rate of lactate production during exercise is unaffected by training (43, 159). These findings suggest that the lower blood lactate concentrations during exercise in the trained state may be due to an increased rate of lactate removal, rather than a reduced rate of lactate production. However, the use of isotopic tracer techniques for the study of lactate metabolism has been criticised on the basis that the majority of the lactate label is lost due to a rapid equilibration between lactate and pyruvate, such that the calculated lactate turnover might more accurately reflect pyruvate rather than lactate flux (174, 214). Given the criticisms associated with the use of lactate tracers various non-tracer methodologies have been used to examine the effects of training on lactate metabolism. One such non-tracer methodology that has been used is a comparison of the effect of training on the rate of blood lactate decline during recovery from exercise, with a more rapid rate of blood lactate decline being interpreted as reflecting an increased rate of lactate removal (15, 52, 57). The

work described in Chapter 3 of this thesis compared differences in the rate of blood lactate decline between endurance-trained and untrained subjects during recovery from supine cycling exercise, and was the first study using this methodology to provide evidence of an increased rate of blood lactate removal in endurance-trained subjects.

In Chapter 4, the validity of interpreting differences in the rate of blood lactate decline during recovery from exercise as reflecting differences in rates of blood lactate removal was examined. The validity of such an interpretation is dependent on whether or not the kinetics of the addition of lactate to the blood by previously active skeletal muscle is affected by training. If the addition of lactate to the blood by previously active endurance-trained and untrained skeletal muscle did not decline at similar rates during recovery from exercise, differences in the rate of arterial blood lactate decline between endurance-trained and untrained subjects would not necessarily reflect differences in the rate of lactate removal. However, the work described in Chapter 4 indicated that training does not affect the kinetics of the addition of lactate to the blood by previously active skeletal muscle. This finding was interpreted as indicating that the difference in the rate of blood lactate decline that was found between the endurance-trained and untrained subjects in Chapter 3, did most likely reflect a difference in the rate of blood lactate removal.

The work in Chapter 4 also examined whether endurance exercise training increases the ability of skeletal muscle to take up plasma FFA during

exercise. Although it is well recognised that the lower blood lactate concentrations during exercise following training are at least partly due to an increased utilisation of fats as a substrate during exercise, there is considerable debate relating to the source from which the additional FFA are derived. Some studies have provided evidence that the additional FFA are drawn from the plasma (81, 119, 200), whereas others have indicated that intramuscular triacylglycerols are the source from which the additional FFA are derived (104, 135). In the work described in Chapter 4, the net uptake of plasma FFA by endurance-trained and untrained forearms was compared during handgrip exercise, and, despite the trained forearms demonstrating a reduced net lactate output at a given work load in comparison to the untrained forearms, there was no difference in net plasma FFA uptake. This finding suggests that any additional FFA utilisation by trained skeletal muscle during exercise, which leads to a reduced net lactate output, is the result of an increased oxidation of FFA drawn from some source other than the plasma.

It has not only previously been shown that training increases lactate removal during exercise (43, 132, 159), and recovery from exercise (see Chapter 3), but that lactate removal, at least in trained rats, is also enhanced at rest (44, 45). Inactive endurance-trained skeletal muscle has been proposed as the site of the increased lactate removal at rest (44, 45), and in Chapter 5, the possibility that inactive endurance-trained skeletal muscle can remove more lactate from the blood than inactive untrained skeletal muscle was investigated. Rates of lactate removal between inactive endurance-trained and untrained forearms were compared during an incremental venous infusion of sodium

lactate. No differences in lactate removal were found between the two groups of forearms, suggesting that inactive endurance-trained skeletal muscle does not remove any more lactate from the blood than untrained skeletal muscle, and that the greater lactate removal previously reported in resting trained rats (44, 45) was due to an increased rate of lactate removal by some tissue other than trained skeletal muscle.

Given that inactive skeletal muscle can act as both a passive sink for lactate, as well as a site of net lactate disposal during exercise (1, 3, 28, 31, 71, 163), but that the work in Chapter 5 found no difference in lactate removal between inactive endurance-trained and untrained skeletal muscle, it was decided (in Chapter 6) to investigate whether the various systemic adaptations which occur in response to endurance training provide an environment during exercise which is conducive to an increased rate of lactate removal by inactive untrained skeletal muscle. To investigate this problem, endurance-trained and untrained subjects performed incremental supine cycling exercise to exhaustion whilst net lactate uptake by inactive forearms was assessed. It was found that the forearms of the untrained subjects removed a significant quantity of lactate from the blood during exercise and recovery, but that the forearms of the endurance-trained subjects did not remove any lactate, and were instead, sites of net lactate production. The differences in lactate metabolism between the forearms of the trained and untrained sujects were explained by differences in the arterial blood lactate concentration. The rate of net forearm lactate uptake was linearly related to the arterial lactate concentration in both groups of subjects, and since the arterial blood lactate concentration was significantly

lower in the TR subjects during exercise, there was less opportunity for the forearm skeletal muscle of these subjects to take up and dispose of lactate from the blood. These findings were interpreted as indicating that training induced adaptations in lactate metabolism by inactive skeletal muscle are related to changes in the arterial blood lactate concentration, and therefore occur as a result of the adaptations in other tissues which serve to reduce the arterial blood lactate concentration at a given exercise intensity.

In summary, it was concluded that the lower blood lactate concentrations at a given submaximal work load in the trained state are the result of two processes:

1) a reduced rate of net lactate production by the trained skeletal muscles, and

2) an increased rate of lactate removal by some tissue other than skeletal muscle.

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# ADDENDUM 1- RESPONSES TO THESIS REVIEW COMMENTS AND AMENDMENTS

During the review process of this thesis, the first reviewer suggested that the degree of Doctor of Philosophy be awarded, whilst the second reviewer indicated that the degree be awarded subject to certain amendments being made. In accordance with University policy, my supervisor, Associate Professor Garry Scroop, indicated in a letter to both Graduate and Research Studies and the second reviewer which of the suggested amendments required a response from myself. Below is a record of the amendments suggested by the reviewer and my responses. Where indicated the amendments have been made in the text of the thesis.

## General Methods

**Reviewer's comment:** More detail of the 'custom-made' isotonic handgrip dynamometer must be given in order that the reader can judge its application. An acknowledgment should have been included, plus a photograph or a diagram, whether or not the candidate is the inventor to the system.

**Response:** A separate addendum (Addendum 2) has now been included in the thesis, which gives details of the design and construction of the handgrip dynamometer.

# Chapter 3

**Reviewers comment:** More volunteers would have reduced the data variance, but the important point is that other possible interpretations (of the data) should be recognised and suggestions for improvements in experimental design made.

**Response:** It is possible that more volunteers would have reduced the variance of the data, but nonetheless, with the number of volunteers used it was still possible to demonstrate a significant difference in the rate of blood lactate decline between trained and untrained subjects. However, I must agree that other possible interpretations should be recognised, in particular the possibility that an increased rate of blood lactate decline might not necessarily reflect an increased rate of tissue lactate removal, but may instead be indicative of a difference in the rate of lactate output by previously active muscle. This question was addressed in Chapter 4, but should also perhaps have been discussed, at least briefly, in the discussion section of Chapter 3. To this end the discussion section of Chapter 3 has been amended to include a discussion of the possibility that the observed differences in the rate of blood lactate decline could have resulted from differences in the rate of addition of lactate to the blood by the previously active skeletal muscle.

The reviewer also suggested (but did not require an amendment) that the experimental design could be improved by having the untrained group perform less exercise so that La(0) would be more arithmetically similar for each of the two groups. I disagree with this suggestion since, as stated in the text, analysis

of the data indicated that the rate of blood lactate decline was independent of La(0) ($r = 0.45$, $P>0.05$). If the rate of blood lactate decline is independent of La(0), manipulation of the blood lactate concentration at the end of exercise should not influence the rate of blood lactate removal.

**Reviewer's comment:** Where/how is it envisaged that the proposed pH induced inhibition of lactate removal by enzyme inhibitory effects occurred; in muscle, kidney or elsewhere?

**Response:** In discussing the negative relationship between pH(0) and $\gamma_2$, and the fact that other studies had shown that many of the enzymes involved in lactate removal are inhibited by decreased pH, I was alerting the reader to the possibility that arterial acidosis could lead to inhibition of enzymes located in the cells of those tissues which regulate the metabolic pathways involved in lactate removal. I intentionally did not suggest a tissue bed in which this inhibition occurs since I had no data to confirm or deny the operation of such a mechanism in the tissue beds in which lactate removal occurs and to do so would be pure speculation.

**Reviewer's comment:** Separate correlations for the relationship between $\gamma_2$ and pH should be provided for each subject group.

**Response:** The data were pooled because there was no statistical difference in the relationship between $\gamma_2$ and pH for the two groups of subjects. I must agree

that since this was not made clear in the text of the thesis pooling the data may have seemed questionable, but since there was no difference in the relationship between $\gamma_2$ and pH for the two groups there is no point in providing two separate correlations. Instead, the text of the thesis has been amended to clarify that there was no difference in the relationship between $\gamma_2$ and pH for the two groups.

**Reviewer's comment:** More awareness of the potential shortcomings in this experimental design should have been made throughout the chapter.

**Response:** From this statement I can only conclude that the reviewer was again referring to the potential shortcomings in experimental design due to the arithmetic differences in La(0) which could have been rectified by the untrained subjects doing less work. I do not believe that any further response is required on my part in order to address this criticism as I have already discussed the reviewer's comments relating to the effect of differences in La(0) on lactate removal rates.

## Chapter 4

**Reviewer's comment:** It is not made sufficiently clear whether for each racquet player the untrained forearm is contralateral to the playing arm.

**Response:** An amendment has been made which clarifies that the untrained forearm was not always contralateral to the playing arm, and was in fact any forearm which demonstrated a peak oxygen uptake of less than 100 μmol·100 $ml^{-1}$·$min^{-1}$.

**Reviewer's comment:** Some sources of variability might have been eliminated if the design of the experiment had allowed a comparison within individuals.

**Response:** Such a design had been used in a previous study published by this author and had been contemplated for use in the present work, but the volume of preliminary testing required to identify individuals who possess such a physiology has in the past proven to be difficult, if not nearly impossible and so was not used in the present study. In any case, with the present design it was still possible to demonstrate a statistically significant difference between the two groups of forearms.

**Reviewer's comment:** The statement made at 4.3.4 regarding the more rapid increase in RER in the untrained forearms must be supported by statistical and/or graphical evidence.

**Response:** In the General Methods section it was stated that the $\alpha$ level for statistical significance was set at $P \leq 0.05$ unless otherwise stated. The

statement referred to by the reviewer (at 4.3.4), that the RER increased more rapidly in the untrained forearms, was based on the finding of a statistically significant interaction effect (at the 0.05 level) between forearm training status and exercise workload. It was assumed that the reader would understand that the author would not state that a particular variable had changed in some way unless a statistical test had been performed which identified such a change. Nevertheless, in line with the recommendation of the reviewer an amendment has been made which makes it clear that a statistical test had been performed which identified the more rapid increase in RER.

**Reviewer's comment:** Concerning the estimation of forearm RER, no statement was made of how this was derived, although one potential source of error (blood pH) was acknowledged. Displacement of $CO_2$ from plasma bicarbonate by local $H^+$ and also the synthesis of new bicarbonate by red cells from local $CO_2$ create a complex set of comparisons when calculating regional RER from arterio-venous blood gas content differences. A fuller and more balanced consideration of these factors and whether the estimation can have useful interpretive value is needed.

**Response:** As indicated by my supervisor, the RER is a standard variable and the calculations used for gas contents are shown in the General Methods section of the thesis. As for the reviewer's comments relating to the displacement of $CO_2$ from plasma bicarbonate by local $H^+$ and the synthesis of new bicarbonate by red cells from local $CO_2$, these effects were considered in

the discussion of Chapter 4. As indicated by the reviewer, the discussion section of Chapter 4 did include a discussion of the fact that calculations of RER could have been influenced by the formation of $CO_2$ from the buffering of $H^+$ by the bicarbonate system. The displacement of $CO_2$ from plasma bicarbonate by local $H^+$ and the synthesis of new bicarbonate by red cells from local $CO_2$ referred to by the examiner are merely statements of the reactions which make up the bicarbonate system already discussed in the text. Bicarbonate formation from $CO_2$ and $CO_2$ formation from bicarbonate are simply the reverse reactions of the same pathway ($H^+ + HCO_3^- \Leftrightarrow H_2CO_3 \Leftrightarrow H_2O + CO_2$). No detailed discussion of the equilibrium nature of the reactions involved in this pathway was embarked upon in the discussion to Chapter 4 since it was assumed that it is widely understood that the reactions can proceed in both directions, and under the conditions which were operative in the experiments conducted in Chapter 4 the equilibrium would have favoured movement of this reaction to the right so no discussion of the reverse reaction was thought necessary. Furthermore, in Chapter 4 no turnover rates of the metabolites involved this pathway were measured, so it was only felt necessary to discuss net effects.

**Reviewer's comment:** The penultimate sentence of the concluding paragraph of Chapter 4 should end, - 'in tissues other than the exercising muscle', and the final sentence should include the following;

- during *20 min* handgrip
- suggests that *if* any additional FFA
- during exercise *it must be* the result

**Response:** The latter two amendments have been made, but the first amendment was not since the handgrip exercise performed was not for a period of 20 min.

## Chapter 5

**Reviewer's comment:** A diagram depicting the fluxes and sites of removal of lactate would be ideal here or in the general conclusions.

**Response:** I agree that such a diagram would be useful, but no data were collected from which to construct such a diagram, the only tissue in which lactate flux and/or removal was measured in the present thesis was skeletal muscle.

## Chapter 6

**Reviewer's comment:** The data in this chapter were apparently collected during the whole body exercise experiments reported in Chapter 3. Although this division of information is acceptable, it should have been volunteered.

**Response:** An appropriate amendment has been made to clarify that the data were collected during the whole body exercise experiments reported in Chapter 3.

**Reviewer's comment:** The very conclusion of net lactate production from non-exercising trained (skeletal) muscle (during leg exercise) must be questioned. Could the higher blood flow through forearm skin of trained subjects have biased the calculation of lactate production from whole forearm to a significant extent.

**Response:** This is not the first study to demonstrate net lactate production by non-exercising forearm muscle during leg exercise. Ahlborg and Felig (1) demonstrated that the arterio-venous difference across inactive forearms became progressively more negative (indicating net lactate output) during prolonged leg exercise, reaching values three times the basal level of lactate output at 3.5 hours. Given that it has previously been shown that inactive skeletal muscle can act as a site of net lactate output during leg exercise, the finding of a net lactate output by inactive skeletal muscle during leg exercise in the present study may not seem so questionable. Secondly, as to whether the higher blood flow through the skin of the trained subjects could have biased the calculation of lactate production from whole forearm; when calculating metabolite fluxes from measurements of limb blood flow and arterio-venous differences, as was done in the present study, it is necessary that the venous blood samples should be representative of the venous drainage from the tissues

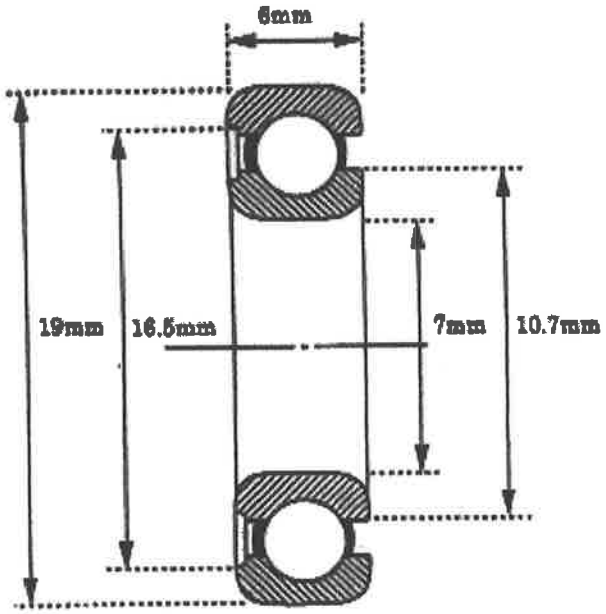
through which blood flow is measured. In the present study total forearm blood flow was measured and, although the venous blood samples were taken from deep forearm veins which predominantly drain forearm muscle, about 10% of the venous drainage may come from the other forearm tissues (148). Therefore, since the venous blood samples taken are representative of the tissue bed through which blood flow was measured the measurements of metabolite fluxes should be valid. No amendment has been made here since the validity of using the forearm as a model for metabolic studies was discussed in the General Methods section of the thesis.

# ADDENDUM 2 - HANDGRIP DYNAMOMETER DESIGN

The handgrip dynamometer consists of a cam/pulley and springs configuration designed to deliver a constant resistance. The dynamometer was designed and constructed by students of the Department of Mechanical Engineering at the University of Adelaide, under the supervision of Dr. G. Tansley.

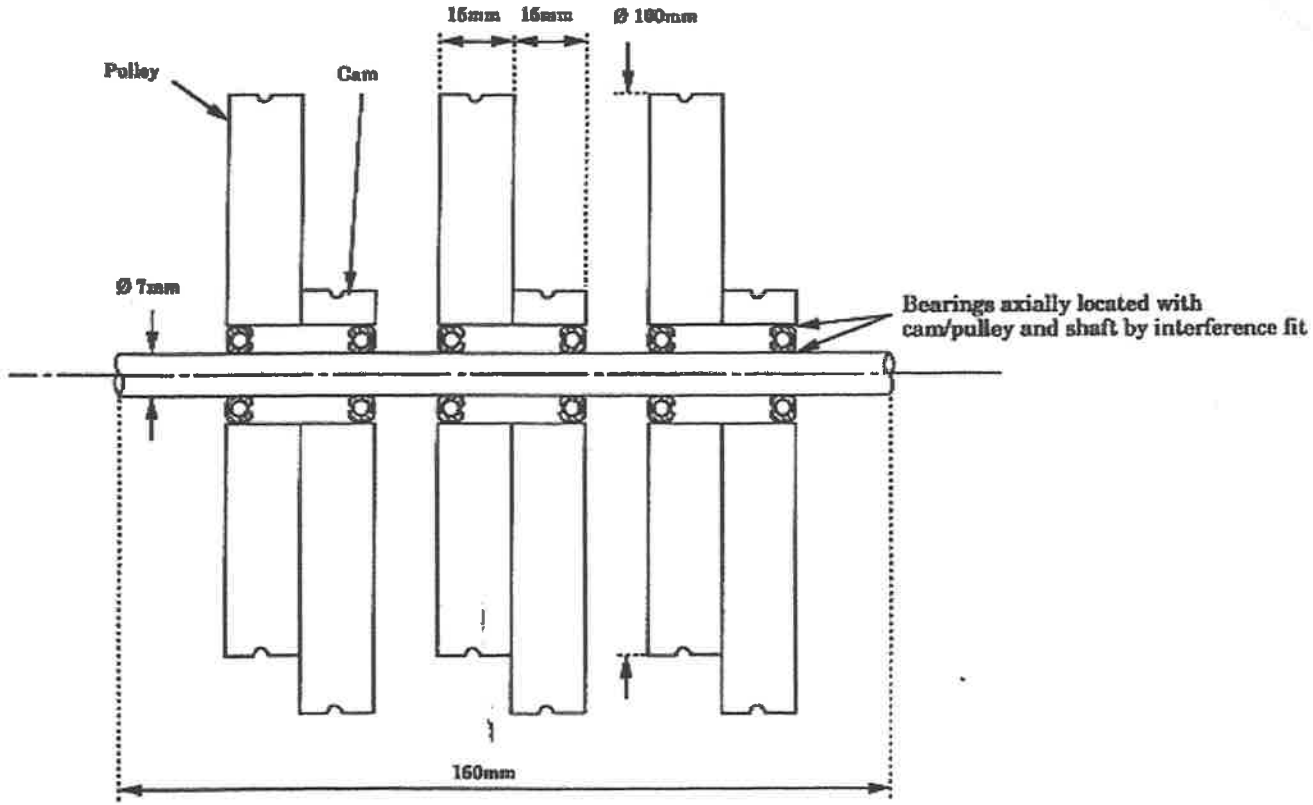
The loading mechanism for the dynamometer consists of three polyvinylchloride (PVC) cam and pulley units mounted on a single shaft of mild steel (160 mm length x 7 mm diameter). The cams and pulleys (100 mm diameter) have a thickness of 15 mm and were manufactured using numerically controlled milling machines in the Mechanical Engineering laboratory of the Department of Mechanical Engineering at the University of Adelaide. For ease of manufacture, the cams were made as separate units from the pulleys and can be pinned together by use of dowels. The profile for the cams was determined using the method outlined in the section 'Calculation of Cam Profile' given below.

The cams and pulleys were mounted on the shaft using double shielded deep groove ball bearings. The dimensions and arrangement of the bearings are given in the following diagrams:




Brg. is supplied lubricated with Lithium based grease. Relubrication is not necessary.

Bearing specifications (cat.no. : SKF 607-2Z)




Schematic layout diagram of cam/pulley arrangement

A spring/cam mechanism provides a constant resistance throughout the range of movement of the dynamometer. The spring/cam mechanism has three identical sections, each providing a load range of 2.5 - 5 kg. Each section consists of two springs, a cam and a pulley. One spring is connected to a stainless steel cable which is pulled by the handle of the handgrip device. The other spring is connected to the cam by another cable. The cam is mounted on the side of the pulley, which in turn has a third cable connecting it to the first cable.

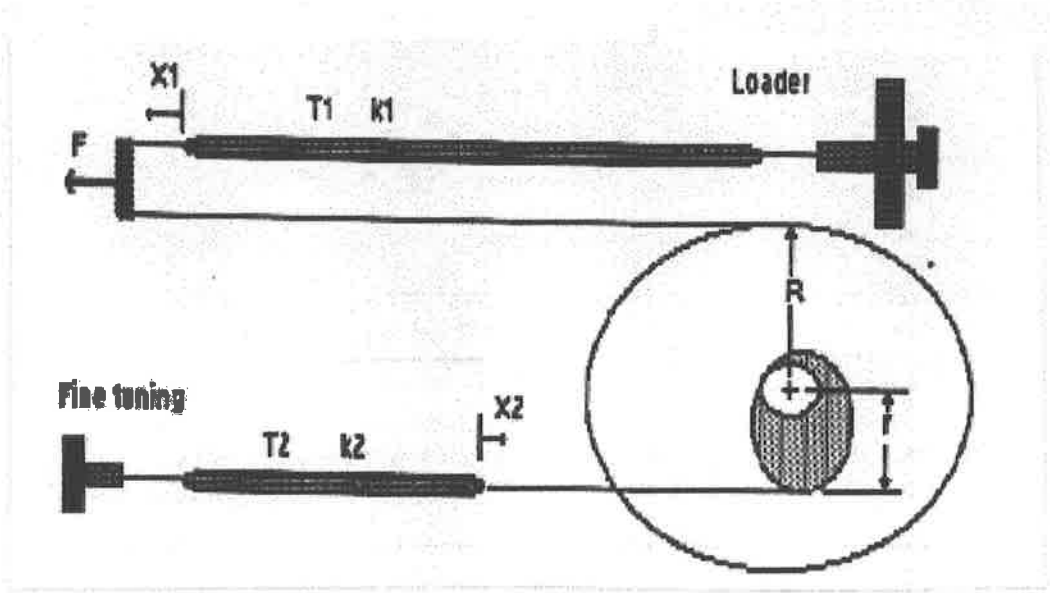
The load experienced at the handle of the handgrip device is the sum of the load produced by the first spring and that from the second spring, the cam and the pulley. As the handle is compressed the first spring is stretched causing the load exerted from that side to increase. At the same time the cam and pulley rotate. Due to the reduced mechanical advantage caused by the rotation of the cam the load exerted from the second side (i.e. the other spring) is reduced. At all times the summation of both sides is always the same, resulting in a constant load being experienced at the handle.

Springs experience constant stiffness (the increase in load/distance extended) throughout their entire extension. This allows the tension to be changed on the first spring by altering its length to obtain different loadings without affecting the constant loading properties experienced at the handle.

A strain gauge annulus was placed in between the main cable and the handle to allow for connection to an external recording device.

## Calculation of Cam Profile

For one of the three cam/pulley units:




The force exerted by the patient is given by the following formula, found using Hooke's law.

$$F = k1 \cdot x1 + T1 + (ks \cdot x2 + T2)r/R$$

where:

R = radius of pulley (50 mm)

k1 = k2 = spring stiffness (0.075 N/mm)

T1 = initial tension in spring 1 (5 [upto 30] N)

T2 = initial tension in spring 2 (19.5 N)

F = load range for cam/pulley unit (2.5 - 5 kg)

$x1 = R \cdot \theta$

Substituting values into the equation yields:

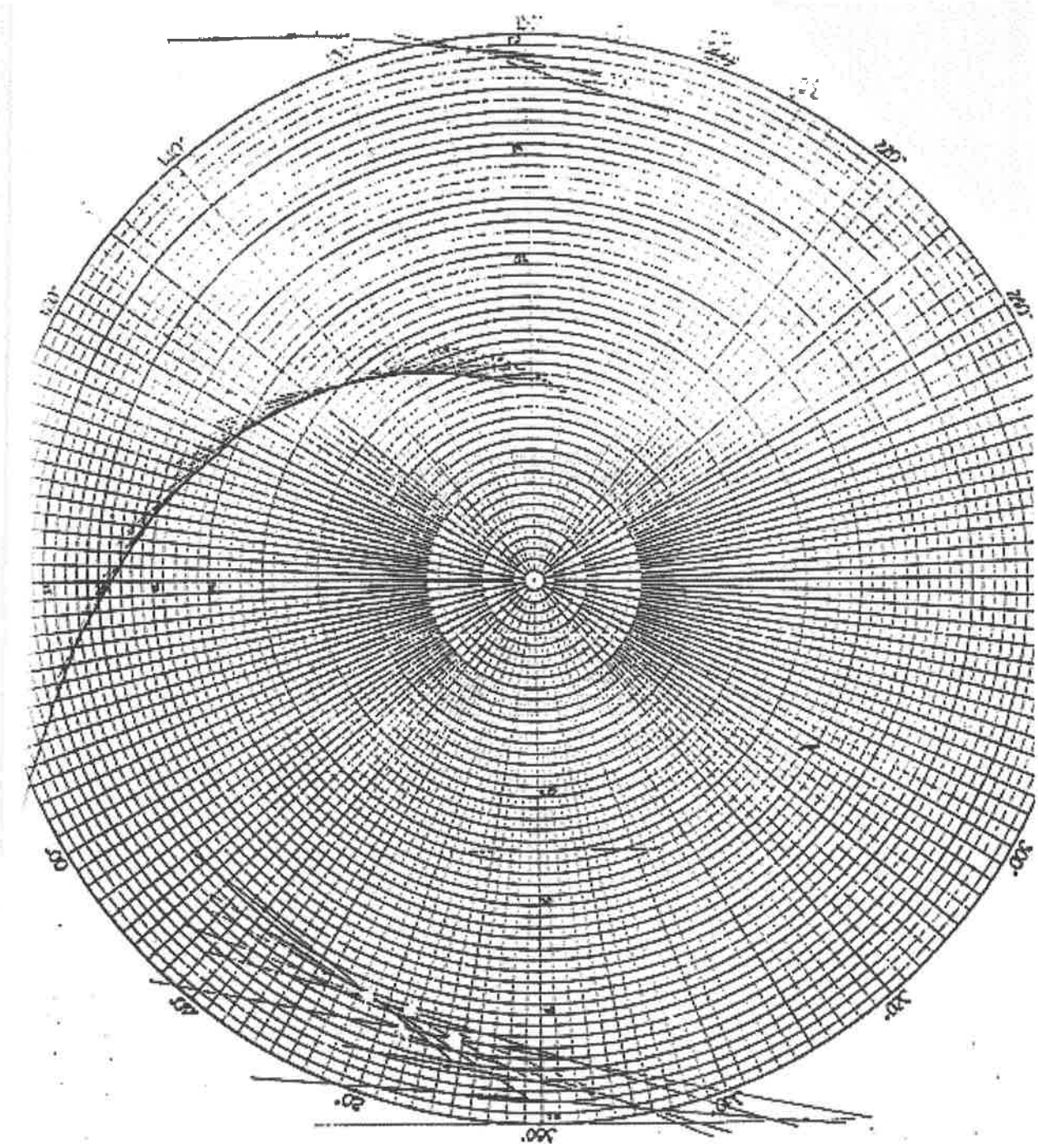
$$24.5 = 50 \cdot 0.075 \cdot \theta + 5 + \frac{r \cdot 19.5}{50} + \frac{r \cdot x2 \cdot 0.075}{50}$$

This is an equation with independent variable $\theta$ and other variables x2 and r. Assuming that the average value for $x2 = \frac{50 + r}{2}$ the formula will then have one variable and one unknown and can be solved for the unknown r.

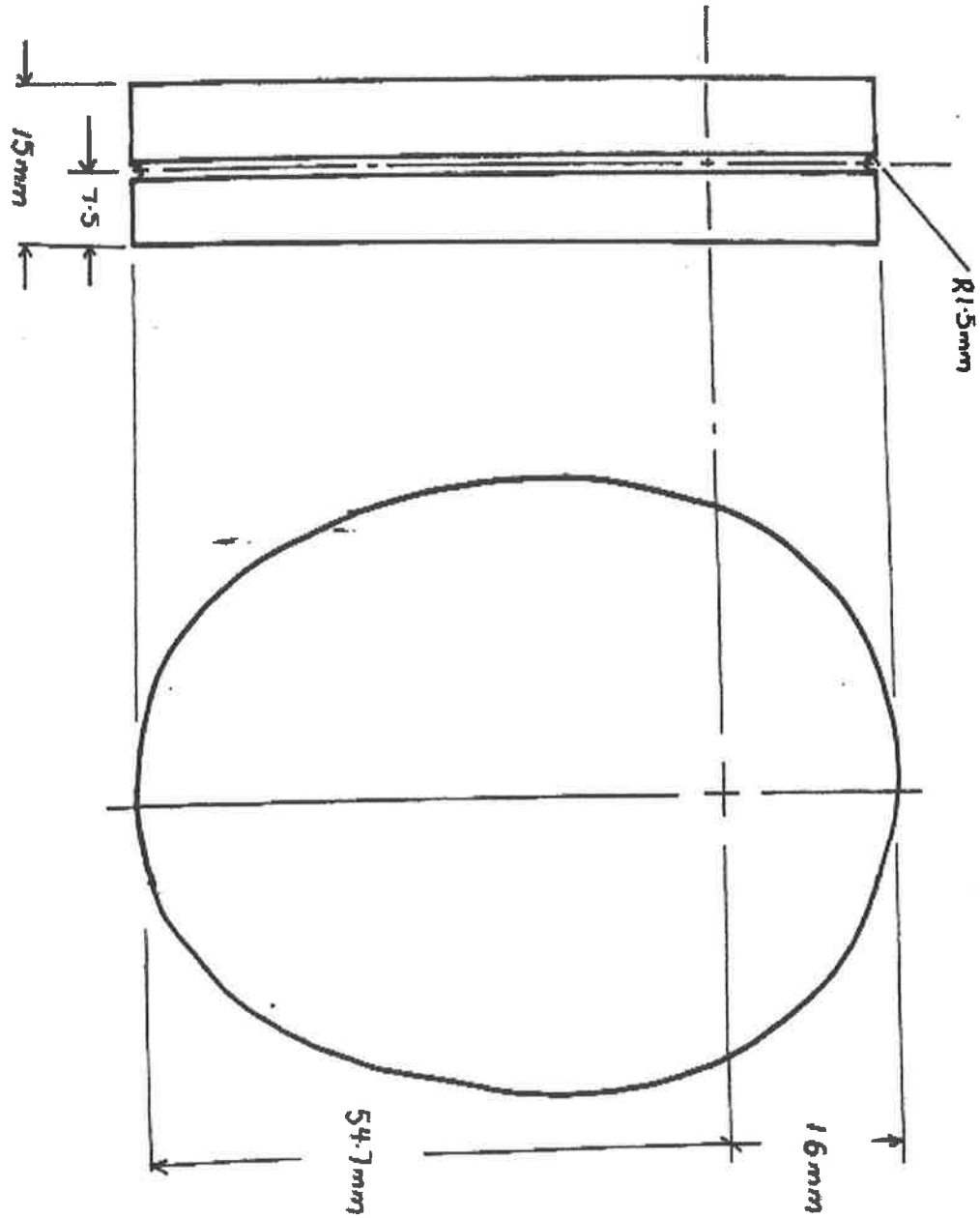
$$24.5 = 3.75 \cdot \theta + 5 + 0.39 \cdot r + \frac{(50 + r)r}{2} \cdot 0.0015$$

$$0 = 0.00075\, r^2 + 0.4275\, r + 3.75\, \theta - 19.5$$

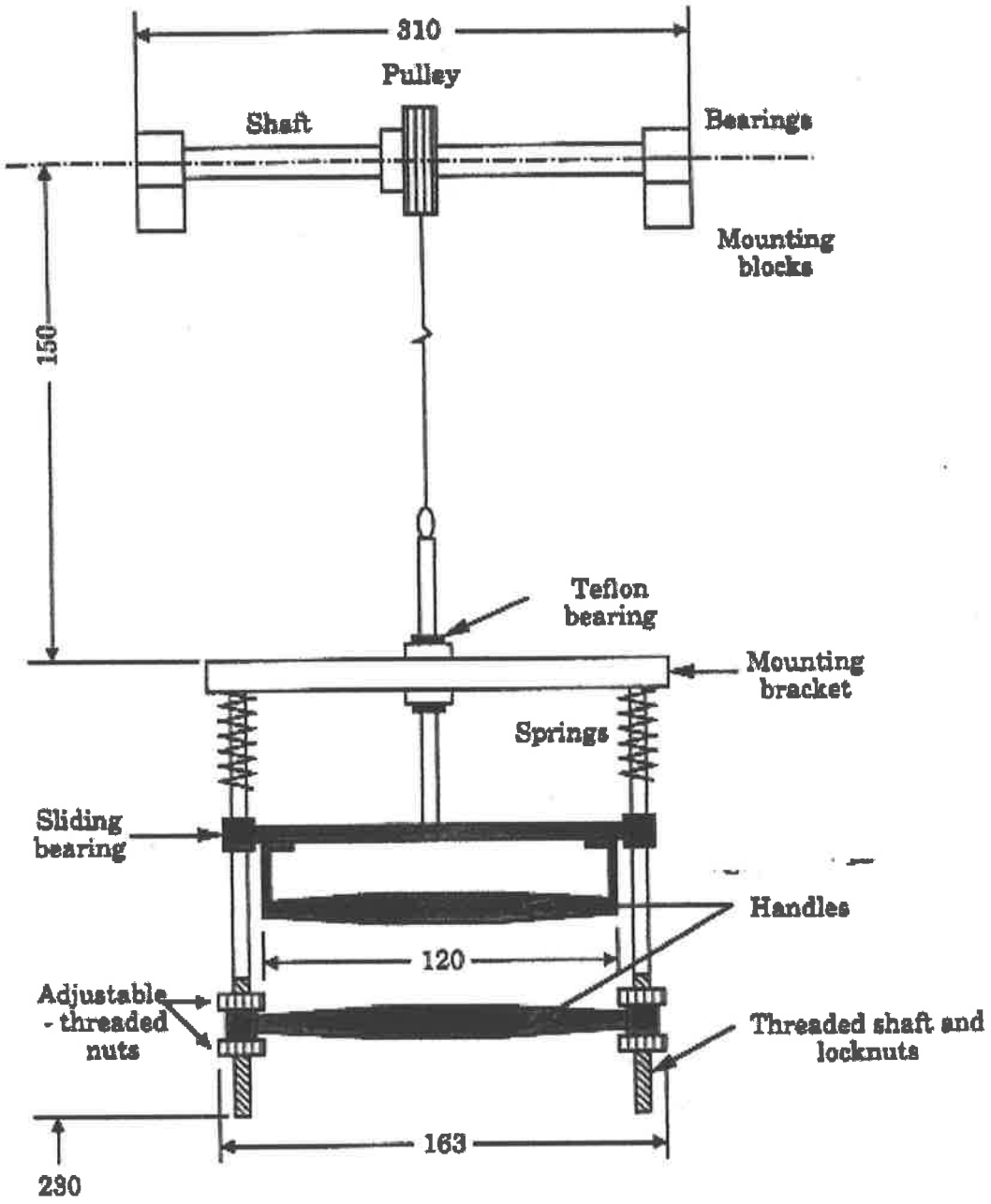
The equation is solved numerically (i.e. finding the values of r for various values of $\theta$). The points are then plotted on a circular graph. From each point on the circular graph a tangent is drawn. The intersection of these tangents form the border of the cam (see next page).



The dimensions of the cam are given in the diagram below:



Diagrams of the handle mechanism and the overall design of the handgrip dynamometer are given on the following two pages.




**Top view of handle assembly** (all dimensions in mm)