Lactate Production and the Redox State of Muscle

A Thesis Submitted for

the Degree of Doctor of Philosophy

in the Faculty of Medicine

by

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ABSTRACT

Opinion is divided as to whether lactate is produced due to oxygen limitation, or whether oxygen ever reaches limiting levels in exercising muscles and lactate produced due to some other effect. This can be examined from knowing that the cardiac output can only supply one-third to one-half of the muscles of the human body when maximally active with an optimal supply of blood. If a small muscle group is exercised simultaneously with a large muscle group a compromised blood and oxygen supply to one or both of the muscle groups will result. In this thesis, the wrist flexors of a single forearm (representing a small muscle group) carried out a series of flexion exercises at easy, moderate, and maximal intensities at first alone, then repeated superimposed on top of bilateral supine cycling (representing a large muscle group). Blood flow was measured in the exercising and resting forearms. Arterialised venous blood, and venous blood effluxing the exercising forearm were also sampled to give an indication of net lactate movement across the working muscle.

There was only non-significant evidence of a reduction in the forearm blood flow as the leg exercise intensity increased. This was attributed to the supine position of the exercise causing greater perfusion of the forearm muscles than would be achieved in an upright subject. The blood lactate concentrations showed a net lactate output from the forearm when exercising alone. As leg exercise intensity increased arterial blood lactate concentration rose and the exercising forearm changed to net lactate uptake. The exercising forearm muscles exhibited net uptake, or no net lactate movement, even when working maximally. This was when the arterial lactate concentration was above 7

$mmol.l^{-1}$, presumably causing the blood-muscle lactate concentration gradient to be towards the muscle.

The fact that lactate could go into the muscle in conditions of maximum metabolic challenge when the blood flow may have been compromised seemed to refute any possibility of lactate production being due to oxygen starvation. This then led to the question of whether the muscle ever becomes oxygen-limited in exercise. The technique of Near Infrared Spectroscopy (NIRS) which can monitor the oxidation state of haemoglobin and cytochrome oxidase non-invasively in tissue was applied to the problem. Cytochrome oxidase is the only point in aerobic metabolism that makes direct use of oxygen, so will be the point at which any shortfall will be most apparent.

NIRS of the forearm muscle was carried out during isometric handgrip exercise in conditions of normoxia, hyperoxia, and hypoxia, with free blood flow to the forearm muscle or with flow occluded using a brachial cuff. No effect was seen on the cytochrome oxidase in the non-occluded bouts of exercise. When under occlusion, and particularly in conditions of hypoxia, the cytochrome oxidase enzyme became more oxidised.

This seems contrary to conventional thinking of oxygen levels becoming reduced as exercise intensity increases. In conditions of occluded exercise the muscle essentially becomes a closed system, and the products of muscle metabolism will build up in the area of metabolic activity. The argument proposed is that this will alter the environment of the aerobic energy generating enzymes away from optimal. This will

reduce the ability of aerobic metabolism either to generate reducing equivalents, or to transport them down the Electron Transport Chain (ETC). It will also change the mitochondrial environment and the mitochondrial membrane potential by increasing the extramitochondrial proton concentration. This will reduce the proton transfer necessary out of the mitochondrial matrix to maintain the driving force on ATP synthetase. These mechanisms will both contribute to reducing the electron flux through the cytochrome oxidase complex causing it to become more oxidised overall.

The oxidation state of the forearm muscle was then monitored during easy, moderate, and maximal dynamic exercise. This caused the cytochrome oxidase enzyme to become more reduced. The energy demand of dynamic exercise is characteristically much higher than that of isometric exercise. This will cause a much greater generation of reducing equivalents, and a greater flux of electrons down the ETC and through the cytochrome oxidase enzyme. The enzyme will then receive more electrons, and become more reduced overall. Simultaneous to the cytochrome oxidase becoming more reduced, the generation of deoxyhaemoglobin increased in roughly equal steps in the three bouts of exercise. Oxygen, from oxyhaemoglobin, must therefore still be present weighing the scales against any possibility of oxygen-limitation causing the results seen.

The initial study looking at the lactate movements across an exercising forearm was then revisited, looking this time at the oxidation state of haemoglobin and cytochrome oxidase instead of blood lactate concentration. Additional evidence was obtained here suggesting blood had in fact been diverted from the arm to the active legs in this and

the earlier experiment. However, there was no significant change in the oxidation state of the exercising forearm muscle as leg exercise intensity increased. In the most severe exercise conditions when the arterial lactate concentration would be highest and the forearm previously exhibited either no net lactate output or actual lactate uptake, there was some evidence of a non-significant fall in reduction/rise in the oxidation state of cytochrome oxidase. If the lactate taken up by the muscle then goes on to be metabolised as presumed, it must do so aerobically and will still generate an electron flux down the ETC. The rising arterial lactate concentration and build up of the products of muscle metabolism were perhaps beginning to affect the mitochondrial environment and increase the cytochrome oxidase oxidation state, but not significantly enough to impede energy metabolism and ATP generation.

The muscle never seemed to be in conditions of oxygen-limitation, and lactate release seemed not to be associated with oxygen supply. If electrons reach cytochrome oxidase, with oxygen in relative abundance they will be able to reduce it to water. The oxidation state of cytochrome oxidase does change in exercise, but seems determined by the rate of electron flow through the enzyme complex rather than by the availability of oxygen.

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AUTHOR'S DECLARATION

All works described in this thesis are my own, and have not been presented as a thesis, or part of a thesis for a degree in this, or any other university.

All of the experiments were performed by me, except where simultaneous blood samples were required from two separate body sites, in which case I was assisted by personnel from the Western Infirmary.

Advice and assistance was given by Mr J. Wilson for exercise testing procedures, from the Electronics Unit of IBLS for general maintenance and repair of equipment and for data analysis, and from the Robertson Centre for Biostatistics, Glasgow University, for statistical advice. All the data analysis and statistical analysis was carried out by me.

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ABBREVIATIONS

Acetyl CoA	Acetyl Co-Enzyme A
ADP	Adenosine DiPhosphate
AT	Anaerobic Threshold
ATP	Adenosine TriPhosphate
a-v O_2 diff.	Arteriovenous oxygen difference
BF	Blood Flow (with various subscripts)
CO_2	Carbon Dioxide
CtOx	Oxidised Cytochrome Oxidase (with various subscripts)
DPF	Differential Pathlength Factor
e^-	Electron
ETC	Electron Transport Chain
FAD	Oxidised Flavin Adenine Dinucleotide
$FADH_2$	Reduced Flavin Adenine Dinucleotide
H^+	Proton
Hb	Deoxyhaemoglobin (with various subscripts)
HbO_2	Oxyhaemoglobin (with various subscripts)
HR	Heart Rate (with various subscripts)
Lac	Blood Lactate Concentration (with various subscripts)
NAD^+	Oxidised Nicotinamide Adenine Dinucleotide
$NADH + H^+$, $NADH_2$	Reduced Nicotinamide Adenine Dinucleotide

NIR	Near Infrared Light
NIRS	Near Infrared Spectroscopy
O_2	Molecular Oxygen
OD	Optical Density
pO_2	Partial Pressure of Oxygen
Ra	Rate of Appearance
Rd	Rate of Disappearance
$\dot{V}O_2$	Rate of oxygen uptake per minute
$\dot{V}O_{2max}$	Maximum rate of oxygen uptake per minute

ACKNOWLEDGEMENTS

I would like to thank the entire cast of "Lactate Production and the Redox State of Muscle" for all their help in allowing me to complete this production. I am particularly grateful to:

First Assistant Director	Prof. Neil Spurway

who replied to a letter from a swimming Biochemist from Darlington to start the ball rolling, and who has been at my side throughout.

Second Assistant Director	Dr. Stan Grant

whose freely-given advice on all matters was very much valued, and helped keep the ball rolling.

Technical Consultants	John Wilson, and the staff of IBLS

between us we somehow managed to keep the show on the road.

Set Design and Construction	The IBLS Electronics Workshop

who deciphered my insane mutterings and made all the gadgets I dreamt up.

Stuntpeople	My subjects

who went through untold torture and still came back smiling.

The Sponsors	Glasgow University Medical Faculty

who provided the essential financial backing through thick and thin.

Entertainment	The Swimming World Dr. Neil Abbot

who kept me sane.

Crew On Location - Darlington	Mum, Dad, Pinta, Jet

for being there, and not changing the locks.

Thanks everyone.

Chapter 1

THEORETICAL INTRODUCTION

ENERGY GENERATION

Energy can be neither created nor destroyed - only transformed from one form to another (first law of thermodynamics). Animals obtain their energy by transforming organic molecules into chemical energy, stored as high-energy phosphate bonds in Adenosine TriPhosphate (ATP). ATP acts as a rechargeable battery. It releases its energy by breaking its high-energy phosphate bonds, forming Adenosine DiPhosphate (ADP) and inorganic phosphate:

$$\text{Adenosine-}\mathbf{P}\text{-}\mathbf{P}\text{-}\mathbf{P} + H_2O \xrightarrow{\text{ATPase}} \text{Adenosine-}\mathbf{P}\text{-}\mathbf{P} + Pi + \text{ENERGY}$$

This is a hydrolysis reaction, catalysed by a group of enzymes called ATPases. Small amounts of ATP are stored in muscle. Once used, it must be reformed. To recharge the battery, ATP must be reformed from ADP (Adenosine DiPhosphate). The greatest amount of ATP is regenerated by aerobic breakdown of carbohydrates, fats, and proteins, in the citric acid cycle. Proteins, fats, and carbohydrates are all sequentially broken down to form Acetyl Co-Enzyme A (Acetyl CoA - Fig. 1.1). Proteins are also broken down to their constituent amino acids and enter the citric acid cycle at various other points. They can then undergo breakdown to yield energy, or be used as precursors for synthesis of different amino acids and hormones. They generally play a very minor role in energy generation compared with fats and carbohydrates however, so will largely be neglected in this review.

Acetyl CoA formed from fat or carbohydrate enters the citric acid cycle, and combines with oxaloacetate to form citrate. This is sequentially broken down in a cyclic process generating CO_2 and pairs of reducing equivalents usually in the form of hydrogen atoms (usually considered as a proton, H^+, and an electron, e^-), and eventually regenerating oxaloacetate. The hydrogen atoms are carried in the form of reduced Nicotinamide Adenine Dinucleotide ($NADH + H^+$, abbreviated here to $NADH_2$), or reduced Flavin Adenine Dinucleotide ($FADH_2$). $NADH_2$ and $FADH_2$ carry the reducing equivalents to the electron transport chain (ETC), embedded in the inner mitochondrial membrane. As a member of the chain receives a reducing equivalent, it becomes reduced, and the donating compound becomes oxidised (an oxidation-reduction, or REDOX reaction). In this manner, the reducing equivalents are carried down the ETC by a series of redox reactions, achieving by many small steps the reaction:

$$H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O$$

There are three major respiratory enzyme complexes in the ETC: the NADH Dehydrogenase Complex, the Cytochrome $b\text{-}c_1$ complex, the Cytochrome Oxidase Complex[1], and two electron carriers: NAD^+, FAD, to transport electrons between the complexes (Alberts *et al.*, 1983; Fig. 1.2).

[1] In this thesis, the cytochrome oxidase complex, or cytochrome oxidase (CtOx) represents the complex of cytochromes a and a_3, and takes the place of other names such as cytochrome aa_3, and cytochrome c oxidase.

The majority of the hydrogen atoms produced are carried by NAD^+, with only one of the five pairs of reducing equivalents generated carried by FAD. The end point of both reduced carriers is the ETC, where they donate their hydrogen atoms ($H^+ + e^-$) and become reoxidised. The electron is transported down the chain and the proton buffered in solution, to be picked up again in the terminal step to reduce molecular oxygen to water. $FADH_2$ and $NADH_2$ are reoxidised by different electron carriers, and so enter the chain at different points (Fig. 1.2). $NADH_2$ reduces NADH dehydrogenase, and so the electron will pass through all three major enzyme complexes. $FADH_2$ reduces ubiquinone, so the electron will only pass through two of the three enzyme complexes (Alberts *et al.*, 1983).

The ETC starts with the regeneration of NAD^+ from $NADH_2$, and ends in the reduction of molecular oxygen to water. Thus, although the whole of the citric acid cycle and the electron transport chain constitute aerobic metabolism, none of the reactions leading to the production of $NADH_2$ or $FADH_2$ makes direct use of oxygen. Only the reactions that regenerate NAD^+ or FAD involve oxygen, and then only directly at the terminal enzyme complex of the ETC, cytochrome oxidase (Fig. 1.2).

These electron carriers are crucial for continuing energy generation. Each component of the ETC initially becomes reduced as it receives an electron, then reoxidised as it passes the electron on to the next member of the chain. The redox potential gives an indication of the 'electron pressure' of a conjugate redox pair (both the oxidised and reduced forms of the compound). A 50:50 mixture of

$NADH_2$:NAD^+ has a redox potential of -320 mV, indicating a strong tendency to donate electrons. The redox potential drops as electrons flow down the ETC, and a 50:50 mixture of H_2O: $½O_2$ has a redox potential of +820 mV, indicating a strong tendency to accept electrons. This is termed a lower redox potential, despite being numerically higher than the redox potential of $NADH_2$:NAD^+, due to its lower tendency to reduce other compounds. Hence the electrons are passed one-way down the chain. The energy released from the high energy electron as it is sequentially passed through each major enzyme complex is used to pump protons across the inner membrane, from the mitochondrial matrix to the intermembrane space. An average of 1.5 protons are pumped out of the mitochondrial matrix by each enzyme complex, for each electron that it transports (Alberts *et al.*, 1983). Each $NADH_2$ or $FADH_2$ molecule donates 2 electrons to the chain. As $FADH_2$ donates its electrons further down the chain compared to $NADH_2$, it will cause fewer protons to be pumped out of the matrix (Fig. 1.2).

The one-way pumping out of protons creates an electrochemical proton gradient across the inner mitochondrial membrane. ATP is synthesised from ADP by ATP synthetase. This forms a transmembrane proton channel that utilises the proton gradient generated by the electron transport chain to drive ATP synthesis. Protons flow through the channel, down the electrochemical gradient, and back into the mitochondria (Fig. 1.3).

The aerobic regeneration of ATP by oxidative phosphorylation of ADP produces the greatest amounts of ATP, but as it contains many steps and enzymatic

reactions (16 steps in oxidative phosphorylation and the ETC, plus the formation of acetyl CoA from the various substrates), it is activated relatively slowly. In exercises lasting longer than 2 minutes, oxidative phosphorylation provides the majority of the ATP. In events lasting less than 2 minutes however, this system has not reached full activity, so other means of ATP regeneration are necessary (Åstrand & Rodahl, 1986e). All three nutrient sources (carbohydrates, fats, and proteins) are used to yield energy in oxidative phosphorylation, but only carbohydrates can be used for faster ATP production.

Fat yields the most ATP per mole (an average of 129 moles of ATP per mole fat), but can only be aerobically metabolised in the citric acid cycle, so has the slowest rate of ATP production. Fat is a much more reduced compound than carbohydrate, so needs more oxygen to be completely oxidised, only yielding 5.6 ATP per O_2. It does however contain more carbon atoms, so can generate more acetyl CoA per mole, and provide more substrate for oxidative phosphorylation. Carbohydrate yields only 38/39 ATP per mole (depending on whether glucose or glycogen is used initially) but being more a oxidised compound, can yield 6.5 ATP per O_2. Therefore if oxygen is limited, carbohydrate is the preferred substrate (Åstrand & Rodahl, 1986e).

Depending on whether glucose or glycogen is the initial substrate, a net yield of 2 or 3 ATP are generated from glycolysis, in the formation of pyruvate. Glycolysis generates ATP without the need for oxygen. In situations of low to moderate energy demand (e.g. longer duration exercises) the pyruvate formed enters the

mitochondria and is then converted to acetyl CoA. This then combines with oxaloacetate in the citric acid cycle forming citrate, and is aerobically broken down in the citric acid cycle. The $NADH_2$ produced during glycolysis is subsequently reoxidised back to NAD^+ in the mitochondria, as it donates its proton and electron to the ETC.

Glycolysis, which utilises only carbohydrate, is the only means of regenerating enough ATP in exercise of significant duration lasting less than 2 minutes. It occurs in the cell cytoplasm, and provides the substrates for aerobic breakdown of carbohydrate in oxidative phosphorylation in the mitochondria. It also has the capability of generating extra ATP separate from the citric acid cycle (Fig. 1.4). Should the energy demand be higher than obtainable from oxidative phosphorylation (e.g. in high intensity exercise lasting less than 2 minutes), the pyruvate can form lactate, and regenerate NAD^+ without the need of the slower citric acid cycle. This allows glycolysis to proceed faster than oxidative phosphorylation as it has many fewer steps, and it generates an extra 2 or 3 ATP per molecule of carbohydrate (from glucose or glycogen respectively, Fig. 1.4):

$$\text{Pyruvate} + \text{NADH} + H^+ \xleftrightarrow{\text{Lactate Dehydrogenase}} \text{Lactate} + NAD^+$$

Pyruvic acid and lactic acid are both quite strong acids with low pK_a values (Pyruvic acid pK_a = 2.5, Lactic acid pK_a = 3.9), so are almost completely

dissociated in physiological situations to pyruvate and lactate. The lactate produced from glycolysis is released from the muscle into the bloodstream, where it is buffered by the bicarbonate buffering system. In extreme exercise, arterial (whole body) blood lactate can increase from its resting value of approximately 1 $mmol.l^{-1}$, to up to 25 $mmol.l^{-1}$ or more (Kindermann & Keul, 1977). However, due to the buffering capacity of the blood, a 10-fold increase in blood lactate concentration only causes a 1.42-fold increase in the blood H^+ concentration (Åstrand & Rodahl, 1986c). The maximum arterial blood pH shift that this produces is rarely greater than 0.6 pH units.

Although in physiological conditions, lactic acid is almost completely dissociated, lactate accumulation itself is not the source of the intracellular acidification observed in anaerobic glycolysis (Gevers, 1977; Hochachka & Mommsen, 1983; Busa & Nuccitelli, 1984; Åstrand & Rodahl, 1986c). The hydrolysis of ATP produces protons at the muscle cell pH:

at pH 7.40 $\qquad ATP + H_2O \rightarrow ADP + Pi + 0.8H^+$

(Hochachka & Mommsen, 1983)

During oxidative phosphorylation, all the products of ATP hydrolysis (ADP, Pi, H^+) are re-utilised. Thus there is no net accumulation of H^+ in aerobic metabolism:

$$\text{glucose} + 38\ ADP + 38\ Pi + 6\ O_2 + 30.4\ H^+ \rightarrow 38\ ATP + 6\ CO_2 + 44\ H_2O$$

(adapted from Busa & Nuccitelli, 1984)

In anaerobic metabolism however, H^+ are only partially re-utilised in ATP regeneration when glycogen is converted to lactate. In humans, at extreme work levels involving maximal anaerobic metabolism, muscle ATP concentrations can fall from initial levels of 5 $mmol.kg^{-1}$ to about 2.5 $mmol.kg^{-1}$ (Åstrand & Rodahl, 1986c; Hochachka & Mommsen, 1983). This drop represents a potential increase in H^+ concentration from 0.1 $\mu mol.kg^{-1}$ to 2.5 $mmol.kg^{-1}$. It is not surprising therefore that there is a high correlation between blood lactate concentration and pH values. The source of protons, and the fall in muscle and blood pH from 7.0 to below 6.4 in the muscle, and from 7.4 to below 7.0 in arterial blood at maximal exercise, is due to the falling ATP concentrations in anaerobic metabolism, and not the dissociation of lactic acid itself.

The third, most immediate means of ATP regeneration is from another high energy phosphate, phosphocreatine (PCr). This is stored with ATP in the muscle, and so is readily available for energy metabolism. It is called upon immediately ATP is used, and reforms it from the ADP generated, using the energy released from the breaking of its own high energy phosphate bond (Spriet, 1995):

$$ATP + H_2O \rightarrow ADP + Pi + ENERGY$$

$$ADP + Pi \xrightarrow{PCr \rightarrow Cr + Pi + ENERGY} ATP$$

Phosphocreatine stores are extremely limited, only acting as significant energy sources for up to 10 seconds in maximal exercise, but creatine can be obtained from the diet. This has led recently to numerous studies looking at creatine supplementation and its effect on performance (e.g. Harris *et al.*, 1992; Greenhaff *et al.*, 1993). Recent evidence has also suggested that anaerobic glycolysis and PCr degradation are actually activated simultaneously at the onset of exercise (Spriet, 1995). The PCr stores are still depleted in approximately 10 seconds however, so reducing the ATP resynthesis capacity and corresponding maximal exercise capacity of the exercising muscle.

REASON BEHIND LACTATE PRODUCTION

The actual reason behind why lactate is produced in the first instance has been the subject of much debate. Since the observation by Hill & Lupton (1923) that its production increases with exercise intensity, much research has occurred, and the topic has formed the basis of several reviews (Brooks, 1985; Davis, 1985; Wasserman, 1986; Katz & Sahlin, 1988; Walsh & Banister, 1988; Wasserman *et al.*, 1990; Brooks, 1991; Spurway, 1992). At first it was viewed as a waste end product, simply serving to cause fatigue due to the lowering of the pH of the

muscle. Hill & Lupton (1923) originally proposed the idea that lactate was produced in muscle due to an inadequate oxygen supply. The observation that lactate only seemed to be produced above approximately 50% $\dot{V}O_{2max}$ (Bang, 1936), and was produced in greater quantities in conditions of reduced oxygen supply to the muscle such as altitude (Lundin & Ström, 1947), isovolemic anaemia (Miller *et al.*, 1973), impaired cardiovascular response from β-adrenergic blockade (Twentyman *et al.*, 1981), or carbon monoxide breathing (Vogel & Gleser, 1972) strengthened the oxygen limitation hypothesis. The early studies led to the concept of an "anaerobic threshold" (Wasserman & McIlroy, 1964). Below the anaerobic threshold (AT) the oxygen supply to the exercising muscle was deemed to be adequate. Above the AT the oxygen supply began to become limiting for the energy requirements of the muscle, leading to the provision of energy by anaerobic metabolism, and lactate production.

In more recent studies, investigators have challenged the idea that lactic acid is simply a waste end product, and that oxygen limited conditions leading to muscle hypoxia is the cause of increased blood lactate. In a recent review, Brooks (1991) discussed his "lactate shuttle" hypothesis, in which lactate produced by glycolysing muscle fibres could be taken up by other muscle fibres with spare aerobic capacity (either within the same muscle, or at a remote site). This built on the growing concept that lactate was actually a useful metabolic intermediate, and an important means of distributing carbohydrate sources. Lactate has the advantage of being a small molecule of low molecular weight ($CH_3CHOHCOO^-$, M.Wt. = 89), of not requiring insulin for transport, and of moving across cell

membranes by facilitated transport (Roth & Brooks, 1990a, 1990b; Brown & Brooks, 1994).

Lactate uptake and utilisation has been shown to occur in the liver, the heart, and both resting and exercising skeletal muscles. It is then either further oxidised to yield the remaining 92% of energy (as only 8% of the potential is used in the formation of lactate), or reform glycogen in glyconeogenesis (Åstrand & Rodahl, 1986c; Gladden, 1989; Lindinger *et al.*, 1990; McDermott & Bonen, 1992). How, and to what extent lactate is used in the muscles is still debated. Hermansen & Vaage (1977) reported 75% of lactate being re-synthesised to glycogen in the rest periods of intermittent exercise. However, Brooks *et al.*, (1973) suggested only a small percentage of lactate was reconverted into glycogen, and the majority was used for energy production directly, being completely oxidised to CO_2 and H_2O. The real situation is likely to lie somewhere between these two extremes. Oxidation may predominate during exercise, and glyconeogenesis in recovery (Åstrand & Rodahl, 1986c; Mazzeo *et al.*, 1986).

Studies have also shown muscles to be sensitive to the rate and concentration of arterial lactate delivery. Resting muscles from both animals (Chin *et al.*, 1991) and humans (Poortmans *et al.*, 1978; Stanley *et al.*, 1986; Buckley *et al.*, 1993; Bangsbo *et al.*, 1995) have displayed lactate uptake correlated with arterial lactate concentration. Exercising human muscles have largely displayed a net release of lactate during exercise (Jorfeldt, 1970; Stanley *et al.*, 1986). Net lactate uptake has only been shown in extended bouts of exercise (15-90 minutes: Stainsby,

1986; Richter *et al.*, 1988; Gladden, 1991; Stainsby *et al.*, 1991; Gladden *et al.*, 1994). This aggress with the hypothesis that lactate is produced from glycolysis early in exercise periods, supplementing ATP regeneration whilst oxidative phosphorylation is 'catching up'. As the exercise continues, the contribution from glycolysis and lactate formation decreases, and aerobic metabolism takes over. If the exercise is at an intensity to enable it to be continued for a prolonged period of time, the rate of net muscle lactate production will fall, and eventually net uptake of blood lactate may occur.

More direct studies on the concentrations of oxygen in muscle have cast doubt on the "oxygen limiting" hypothesis. The most sensitive index of mitochondrial O_2 availability has been suggested to be the reduction state of the $NADH_2$:NAD^+ conjugate redox pair, an increase in mitochondrial $NADH_2$ denoting a state of O_2 deficiency (Chance, 1976). Jöbsis & Stainsby (1968) followed the oxidation-reduction level of this conjugate redox pair fluorometrically in the gastrocnemius and gracilis muscle of the dog. They showed the NADH consistently became oxidised during twitch activity, when lactate production would be occurring. Graham & Saltin (1989) looked at the oxidation state of human vastus lateralis muscle during cycling up to $\dot{V}O_{2max}$. They inferred the mitochondrial redox state, and the $NAD^+/NADH_2$ ratio from the mass action ratio of the glutamate dehydrogenase reaction:

$$\text{L-Glutamate} + NAD^+ \leftrightarrow \alpha\text{-ketoglutarate} + NH4^+ + NADH_2$$

$$\text{mass action ratio} = \frac{[NH_4{}^+][\alpha - \text{ketoglutarate}]}{[\text{L} - \text{glutamate}]}$$

$$\left(\propto \frac{NAD^+}{NADH_2} \right)$$

They showed that this ratio rose in exercise up to maximal intensities in humans, while large quantities of lactate were being produced. From this they concluded muscle can produce large quantities of lactate without being hypoxic. They did admit however that their data, whilst agreeing with some studies, was in direct contrast to others using different techniques to measure total NAD^+ and $NADH_2$. They attributed this difference to problems differentiating the mitochondrial and extramitochondrial compartments. Further work seems necessary to resolve these differences.

An alternative approach was that of Connett *et al.*, (1984) who measured myoglobin oxygen concentrations directly. They showed that in isolated dog gracilis muscle contracting at intensities up to its own, local $\dot{V}O_{2max}$, and producing sufficient lactate to elevate concentrations in the muscle to 9.5 $\mu mol.g^{-1}$ wet weight, the muscle myoglobin oxygen concentrations never fell below 1.0 - 2.0 Torr. They had concluded that the lower limit for oxygen to become limiting in mitochondria was approximately 0.5 Torr, so lactate was being produced at several times the limiting values of oxygen tension. Lactate is also

produced in resting conditions when oxygen supply can not be limiting, to an arterial blood concentration of approximately 1 $mmol.l^{-1}$, and in low intensity exercise. This whole body resting blood lactate concentration is not a constant value however, but seems to vary with the nutritional status of an individual. Just after a meal, hepatic glycogen levels are high, and hepatic lactate displays net output. This will cause the whole body blood lactate level to rise. After a period of fasting, when the hepatic glycogen levels are low, net hepatic lactate uptake will be evident, and the arterial blood lactate will fall (Wasserman *et al.*, 1991).

However, some groups still argue that the lactate increase is consequent upon a change in the redox state of the muscle, consistent with lactate production being oxygen dependant. A major concern of studies using isolated muscles of anaesthetised animals is whether the $\dot{V}O_{2max}$ generated from electrical stimulation of the muscle group comes anywhere close to "true" $\dot{V}O_{2max}$ of the muscle when used by a conscious animal during maximal exercise (Hoppeler *et al.*, 1987). An acutely exercising conscious animal generates a mean arterial pressure (hence pressure available to perfuse the vascular bed) considerably higher than in an anaesthetised animal. This, combined with the profound hormonal adjustments affecting substrate availability to working muscles in whole animals, may result in the intensity of exercise in the studies of Connett and co-workers (e.g. 1984), simply not being intensive enough to achieve oxygen-limiting conditions.

Concentrations of lactate, pyruvate, and the lactate/pyruvate (lac/pyr) ratio have been used to give indications of the redox state, or oxygen limitation in muscle.

Studies in humans using these metabolites have shown a reduction in the oxidation state beginning at the AT (Wasserman & Koike, 1992). Katz & Sahlin (1987) measured human muscle concentrations of lactate and pyruvate, and showed an increase in the lac/pyr ratio during exercise breathing a hypoxic gas mixture (11% O_2 in N_2). The whole muscle $NADH_2$ concentration has been shown to fall in low intensity exercise, with no corresponding increase in the lac/pyr ratio (Sahlin *et al.*, 1987). Sahlin and co-workers suggest this is consistent with the respiratory chain being stimulated to a greater extent than the formation of $NADH_2$ at low intensities. At higher intensities, corresponding to 75% and 100% $\dot{V}O_{2max}$ (above AT), the $NADH_2$ concentration increased, with a corresponding increase in the lac/pyr ratio. This, they maintain is consistent with a limited availability of oxygen in parts of the muscle at the higher intensities, affecting the re-oxidation of $NADH_2$.

Wasserman & Koike (1992) hypothesised that below the AT, the oxygen supply to the muscle was not limiting, so reducing the oxygen delivery to the muscle would have no effect. In exercise at levels above the AT, the muscle is in conditions of oxygen limitation, so changing the muscle oxygen supply would affect lactate production. Many studies have shown lactate production to be elevated in hypoxic conditions (Hughes *et al.* 1968; Linnarsson *et al.* 1974; Hogan & Welch, 1984; Katz & Sahlin, 1987). Wasserman & Koike reduced the oxygen content in blood perfusing the muscles by increasing the carboxyhaemoglobin levels. They also showed an increase in blood lactate concentrations, but it only occurred above the AT (Wasserman & Koike, 1992).

In answer to studies showing apparently ample levels of oxygen in contracting muscles when lactate is increased, Wasserman *et al.*, (1986) suggested a non-uniform capillary pO_2 within the muscle. This they postulated could result in anaerobic metabolism in some muscle fibres, accounting for the lactate increase despite adequate mean capillary pO_2 values. They concluded this from studies measuring arterial blood lactate and pyruvate. They showed that lactate, pyruvate, and the lac/pyr ratio all increase in a threshold manner, and the lac/pyr ratio abruptly decreased on cessation of exercise. This they attributed to a rate limiting re-oxidation of $NADH_2$ above a threshold point, arising from a more reduced intracellular redox state due to oxygen limiting conditions.

Wasserman *et al.* (1986) inferred changes in the muscle from changes seen in blood metabolites. Blood metabolites usually closely reflect muscle changes and have long been used to develop models to describe the intramuscular response (Karlsson, 1971; Knuttgen & Saltin, 1972; Sahlin *et al.*, 1976, Wasserman & Koike, 1992), but Connett *et al.* (1984) showed in isolated, perfused dog gracilis muscle, an increase in muscle lactate which did not cause an increase in blood lactate. This result fits in well with the lactate shuttle hypothesis (Brooks, 1991), of lactate capable of being produced from glycolysing muscle fibres, and taken up by muscle fibres with spare oxidative capacity, possibly within the same muscle. This theory also agrees with studies showing lactate production from tissues other than the exercising muscles during intense exercise, possibly due to elevated catecholamine levels in the blood (principally adrenaline, Stainsby *et al.*, 1985; Brooks *et al.*, 1991; McDermott & Bonen, 1992), and/or to reduced blood flow

to resting muscle and visceral organs as exercise intensity increased, diverting blood predominantly to the exercising muscles.

In summary, the topic is still one of much debate. However, the pattern of lactate increase in arterialised venous blood seems well accepted, and the brief account given by Wasserman *et al.* (1986) seems typical. For low to moderate work intensities, steady-state blood lactate is not altered. At heavier workrates, a threshold may be exceeded in the muscles, and there is a sustained, small increase in the blood lactate concentration. The blood lactate concentration depends on the rate of appearance (Ra) and rate of disappearance (Rd). At exercise intensities that do not cause blood lactate to exceed a concentration of 4-5 $mmol.l^{-1}$ (OBLA), Rd will eventually catch up with Ra, and a steady state condition results. Fatigue may eventually occur due to depleted muscle glycogen stores. At higher workrates, Rd will be unable to catch up with Ra, so the blood lactate concentration will continue to rise until exhaustion, possibly due in this case to decreased intramuscular pH, rather than hypoglycaemia.

Muscles show net production of lactate above approximately 50% $\dot{V}O_{2max}$. Rather than being a waste metabolite, lactate may be an important carbohydrate source, able to be taken up by muscles. Studies looking at oxygen levels in the muscle have shown that they are several times greater than limiting values when lactate is produced. These studies suggest lactate is a poor indicator of oxygen availability. In contrast, studies looking at indicators of muscle redox state have shown an increase in $NADH_2$ when lactate is produced, consistent with rate

limited oxidation and removal, and oxygen limited conditions. Studies reducing the oxygen supply to muscles show elevated lactate production. Studies increasing the muscle oxygen supply show reduced lactate production. These all suggest that oxygen supply and lactate production are still mechanistically linked.

LIMITATION TO $\dot{V}O_{2max}$

The term "maximal oxygen intake," meaning how much blood the lungs can aerate, the heart pump to the active muscles, and the muscles use, had first been used to quantify aerobic fitness by Hill & Lupton (1923). But research into what actually limits the maximal oxygen uptake of an exercising individual has produced little consensus. Opinion has swayed between central factors such as the pumping capacity of the heart and the capacity of the lungs to aerate the blood being the limiting factor, and peripheral factors such as muscle capillarisation and mitochondrial volumes. Based on the fact that trained athletes have a lower heart rate than untrained individuals when performing the same activity, and that training reduces the heart rate at a particular intensity of work, but has little effect on maximum heart rate (Christensen, 1931) it was initially assumed that the heart was the limiting factor for maximal aerobic capacity. Other work in the 1950's strengthened this assumption. Heart size, particularly atrial and ventricular volumes were shown to be enlarged in endurance athletes, and closely correlated with performance (Saltin *et al.*, 1968). These factors contributed to an enlarged end-diastolic volume. Arterial desaturation was generally not thought to be

significant (Ekblom & Hermansen, 1968), so the pump capacity of the heart alone was thought to be the sole determinant of aerobic capacity.

However, in the 1970's and early 80's, studies were published suggesting that peripheral factors could be the limits to maximal aerobic capacity. Skeletal muscle was shown to be very adaptable to aerobic training (Saltin *et al.*, 1976). Training was shown to produce an increase in capillarisation, mitochondrial volume, and oxidative enzymes in muscles. A significant increase in maximal oxygen uptake in a trained leg, compared to no change in contralateral untrained leg began to suggest adaptations in the muscles had to occur before extra oxygen could be extracted by the muscles from the blood.

The current view is swinging back to the initial hypothesis of central limitation (Saltin & Strange, 1992). Muscular adaptations to training seemed to be many times greater than the improvement in $\dot{V}O_{2max}$ (Gollnick *et al.*, 1973). Studies were published showing two-legged training could cause an increase in the maximum oxygen uptake in the untrained arms, illustrating that increases in oxygen uptake could occur in untrained muscle. The greater cardiac output of the heart caused by the leg training was postulated to cause greater perfusion of the upper body, enabling the muscles to extract more oxygen from the greater volume of blood (Clausen *et al.*, 1973). Di Prampero (1985) calculated the metabolic and circulatory limitations to $\dot{V}O_{2max}$, based on the O_2 path from the environment to the muscle being made up of 3 resistances:

RQ - O_2 transport

Rc - capillary cross-section

Rm - succinate dehydrogenase activity

He showed that in large muscle group exercise (two-legged exercise) 75% of the $\dot{V}O_{2max}$ is determined by the central oxygen transport factor. In smaller exercising muscle groups (one legged exercise) about 50% of $\dot{V}O_{2max}$ is determined by the O_2 transport, the remainder being split equally between the two peripheral factors. The limits to $\dot{V}O_{2max}$ therefore seem to be divided between central and peripheral factors, but in whole body exercise, central limitations predominate.

THE COMPETITION FOR BLOOD

In 1968, Mellander & Johansson proposed on the basis of post exercise measurements with venous occlusion plethysmography, that 40-60 $ml.100ml^{-1}.min^{-1}$ was the maximum blood flow possible in skeletal muscle. Therefore 18 $l.min^{-1}$ of blood was needed to perfuse fully dilated skeletal muscle beds in all major muscle groups. This tied in well with cardiac outputs of 20-25 $l.min^{-1}$ in average healthy individuals.

More recently however, Secher *et al.* (1977) superimposed arm exercise onto continuing leg exercise, and observed a reduction in leg blood flow. They suggested that the maximum cardiac output could not optimally perfuse all the skeletal muscles of the body when they were maximally active. The vasoconstriction in the leg blood flow possibly occurred to maintain the blood pressure, which might have dropped if the heart had continued to supply all the exercising muscles with blood at full rates of flow.

Work then started to find the maximum capacity for perfusion of human skeletal muscle. New techniques looking at muscle blood flow more directly such as thermal or dye dilution, or using radioactive microspheres, showed maximum flow rates during exercise of over 200 $ml.100ml^{-1}.min^{-1}$, with peak flows of over 300 $ml.100ml^{-1}.min^{-1}$ possible (Anderson & Saltin, 1985; Saltin, 1986; Rowell *et al.*, 1986; Waaler *et al.*, 1987; Walloe & Wesche, 1988). The pumping capacity of the heart was now thought not to be sufficient to meet the demands of the skeletal muscle mass in whole body exercise. With a maximum muscle blood flow of 200 $ml.100ml^{-1}.min^{-1}$, and assuming an average muscle mass of 30 kg, (McArdle *et al.*, 1994) in whole body exercise at maximal capacity, the muscles would require a cardiac output of:

$$200\ ml.100ml^{-1}.min^{-1} \text{ for } 30\ kg \text{ muscle mass}$$

$$= 60\ l.min^{-1}.$$

Elite endurance athletes who have cardiac outputs of 35 $l.min^{-1}$ or more, could supply half of their muscles optimally with blood. Average individuals with a cardiac output of 20-22 $l.min^{-1}$ would fully tax their heart in activities requiring more than a third of their muscles to be maximally active.

Reductions in blood flow have been seen however in limbs exercising well below $\dot{V}O_{2max}$ (Secher *et al.*, 1977), but the reason that this occurs at submaximal levels of exercise is unclear. The study by Secher *et al.* (1977) superimposed leg exercise upon arm exercise, and arm exercise upon leg exercise. They saw a reduction in blood flow in the legs on adding arm exercise, with a simultaneous increase in oxygen arteriovenous difference (a-v O_2 diff.) at all submaximal levels. The increase in a-v O_2 diff. seemed independent of the severity of arm exercise. The reduction in blood flow eliciting an increase in oxygen extraction could simply be a safety mechanism triggered by the body in large muscle group activity. Causing vasoconstriction before it is theoretically required will allow a significant 'margin of error' leaving spare cardiac output free to go for example to the skin to facilitate transfer of the heat generated by the extra muscle activity. If the muscles are capable of increasing oxygen extraction, part of the oxygen required for an activity will come from this greater extraction when a second muscle group is recruited. Increasing the active muscle mass results in decreased blood flow in the exercising muscles (Klausen *et al.*, 1972; Secher *et al.*, 1977; Saltin, 1985). This will increase the Mean Transit Time (MTT) of the red blood cells in the muscle, allowing greater oxygen extraction to take place, and so to reduce the cardiac output theoretically needed to supply both muscles. The increase in

capillarisation in trained muscle may only serve to increase the MTT of the red blood cells in the muscle to facilitate an increased a-v O_2 diff. in trained athletes (Saltin, 1985). On this premise, it will not be until very extreme intensities of exercise that a true 'oxygen starvation' situation will be attained, and both cardiac output (central) and oxygen extraction (peripheral) become limiting.

$\dot{V}O_{2max}$ tends to be related to the amount of active muscle mass. Combined leg+arm exercise gives slightly higher values than leg only exercise when cycling, and approximately equal to uphill treadmill running (Secher *et al.*, 1974; Bergh *et al.*, 1976; Åstrand & Rodahl, 1986d). The $\dot{V}O_{2max}$ elicited during combined leg+arm exercise was however much less than the mathematical sum of the individual arm and leg $\dot{V}O_{2max}$ values. The difference between the $\dot{V}O_{2max}$ values was only 4% on average, but the maximal power outputs attained during combined exercise were 18% greater than that achieved during leg only exercise. The extra power output must therefore have come from muscles receiving compromised blood flow and oxygen supply.

Part of this thesis attempts to recreate this potential "oxygen starvation" situation in whole body exercise. A small muscle group, exercising alone, will not challenge the full cardiac capacity, so will receive an optimum supply of blood. As the exercising muscle mass increases, such as when superimposing arm cranking upon upright cycling (Secher *et al.*, 1977) and when comparing the knee extensors of one leg to bilateral upright cycling (Saltin, 1985), the blood flow in the muscles

will decrease, and the oxygen extraction must increase to compensate. By superimposing a larger muscle mass exercising at increasing intensities upon a smaller muscle mass, at some point it is hypothesised that the blood flow and oxygen extraction in the smaller muscle group will become insufficient to provide an adequate oxygen supply to the exercising muscle, and a state of oxygen starvation will result.

NEAR INFRARED SPECTROSCOPY (NIRS)

The use of optical spectroscopy as a physiological tool began early this century, when Hartridge & Hill (1914) reported the near infrared absorption bands of haemoglobin. This was then developed to monitor haemoglobin saturation. The potential of optical spectroscopy as a possible method of examining tissue respiration was not considered until 1955, when Chance & Williams examined the optical difference spectra, between 300 nm and 650 nm, of isolated mitochondrial suspensions. This work was very limited in its potential to measure in-vivo tissue oxygenation, as visible and ultraviolet light (wavelengths less than 700 nm) fail to penetrate more than approximately 1 cm of tissue.

Jöbsis (1977) was the first to address this problem. Near infrared (NIR) light (wavelengths 700-1000 nm) has a relatively high penetration, and can be detected through up to 8 cm of tissue when measured with a suitably sensitive spectrophotometer. The NIR absorption bands of the chromophores (light

absorbing compounds) haemoglobin and cytochrome oxidase had previously been accurately measured (Horecker, 1943; Wharton & Tzagoloff, 1964). Jöbsis showed the possibilities of NIRS by measuring the attenuation spectrum of NIR light across a cat's head. This first data had limited use however, as all concentration measurements were relative, and not absolute (i.e. they were concentration changes from one point in time relative to another). The measurements of haemoglobin and oxidised cytochrome oxidase were also in arbitrary units, as there was no simple reference cuvette against which tissue transmission measurements could be compared. The optical pathlength necessary to calculate absolute concentration changes was also unknown due to the multiple scattering of light in tissue and subsequent increase in pathlength. The early pioneering work of Jöbsis and co-workers (Jöbsis, 1977; Jöbsis *et al.*, 1977; Jöbsis, 1980; Jöbsis *et al.*, 1987; Jöbsis-Vandervliet *et al.*, 1988) can also be subject to some criticism regarding their unwillingness to publish exact details of their approach and methods of NIRS data analysis. Cope (1991) has recently developed the technique of NIRS into something more powerful and clinically acceptable. In his Ph.D. thesis, he formulated an algorithm based on the underlying principles of light absorption, which took into account the scattering properties of tissue, and allowed calculation of the changes in tissue chromophore concentration from optical attenuation measurements of NIR light.

The absorption and scatter of near infrared light in tissue depends on the wavelength of light and the tissue illuminated (Cheong *et al.*, 1990). Within any section of tissue, there are absorbers whose concentration varies with oxygenation

status (oxyhaemoglobin - HbO_2, deoxyhaemoglobin - Hb, and oxidised cytochrome oxidase - CtOx), absorbers whose concentration is essentially fixed (such as melanin and lipid), and scattering particles (e.g. bone, and any area in which the NIR light passes through regions of mismatched refractive indices), all of which serve to increase the observed attenuation of NIR light. The amount of light absorbed by a specific chromophore (light absorbing compound) is also dependant on the wavelength of incident light. This wavelength-dependant absorption is described by the absorption spectrum of the compound, and from this a specific extinction coefficient is calculated, expressed as a function of wavelength. Absorption of light in a clear medium is expressed by the Beer-Lambert law:

$$A = \log\left(\frac{I_o}{I}\right) = \alpha\, C\, d$$

where A = absorbance in optical density (OD) units,

I_o, I = incident and transmitted light intensities,

α = specific extinction coefficient of the absorbing compound, measured in $\mu molar^{-1}.cm^{-1}$,

C = the concentration of the absorbing compound in the solution, measured in $\mu molar$,

d = distance between the points where light enters and leaves the tissue, the geometrical pathlength, measured in cm.

NIRS of tissue, however, is complicated by tissue being a highly scattering medium. In tissue illuminated with NIR light at an interoptode spacing of 5 cm, the loss of light intensity is approximately a factor of 10^8 i.e. for every one hundred million photons incident on the tissue, an average of one photon will emerge for detection (Elwell, 1995). Scattering compounds do not as such absorb light, but simply change the direction of the NIR photons, increasing the apparent pathlength through which the light must travel before it reaches the detector. Tissues of the body all scatter NIR light to different extents, depending on their micro- and macroscopic contents. Broadly speaking, approximately 80% of the total attenuation of NIR light in tissue is due to scattering, and the remaining 20% due to absorption (Elwell, 1995). The Beer-Lambert relationship must then be modified to account for this effective increase in pathlength, by including a pathlength factor, B:

$$A = \alpha C d B$$

The change in attenuation can then be converted into a change of concentration, providing the geometrical pathlength, d, and the differential pathlength factor, B, remain constant:

$$\Delta A = \Delta C \alpha d B$$

As different tissues scatter light to different degrees, measurements of Differential Pathlength Factors (DPF) have been made on several tissue types commonly used in NIRS (Elwell, 1995):

Forearm	DPF = 3.59
Calf	DPF = 4.65
Cranium - Adult	DPF = 5.93
Cranium - Neonate	DPF = 3.71

The calculation of light transport in tissue is by no means simple, and various mathematical models such as Monte Carlo modelling, and finite element modelling have been used to describe its effects. The details are beyond the scope of this thesis, but are dealt with in detail by other authors (Cope, 1991; Arridge *et al.*, 1992; van der Zee *et al.*, 1992; Hiraoka *et al.*, 1993).

Only the compounds whose absorption characteristics vary with oxygenation status (HbO_2, Hb, CtOx) are of interest in this thesis. Of the other compounds, whose presence simply adds to the total light attenuation, water is the most significant. Below 700 nm NIR light is strongly attenuated by tissues such as skin and bone and, above 900 nm, it is strongly absorbed by the water itself. This leaves a narrow 'window' of optical transparency in which spectroscopic measurements of human tissue can be made. The water content of the body is approximately 79% at rest (Woodard & White, 1986), and can fall by as much as

6-10% over a severe endurance event such as a marathon (Wilmore & Costill, 1994). Water loss would have two effects on the NIRS signal:

1) a lower absorption coefficient, due to less absorbing medium being present,
2) a higher scattering coefficient (increasing apparent absorption coefficient), due to increased solute concentrations.

These effects will act in opposite directions, and will be negligible for the haemoglobin signal, and minor (maximum 0.2 μM decrease due to a lower absorption coefficient, and 0.2 μM increase due to a higher scattering coefficient, for a 10% change in water content) for the cytochrome signal (Cope, personal communication). Water loss over the course of an experiment in this thesis was not thought to be severe, so was assumed not to affect the NIRS signal significantly.

Lipid also adds to the total attenuation of NIR light. In this thesis, the forearm was the site of NIR monitoring, and all subjects were fit and regularly active, so subcutaneous fat was not considered significant. Melanin, another NIR light absorbing compound, was also not considered significant in this thesis, as no non-Caucasian subjects participated in the NIRS studies.

At one wavelength, the modified form of the Beer-Lambert equation can only give information about the changes in one chromophore. The same number of different wavelengths as components to be resolved is a minimum requirement in NIRS.

When this is the case, three simultaneous equations are formed from the changes in absorption, and the changes in the chromophore concentrations are calculated from the solution. The Hamamatsu NIRO-500 Near Infrared Spectrophotometer used in this thesis calculates the changes in the concentrations of HbO_2, Hb, CtOx from the absorption of 4 separate wavelengths of NIR light. In this case, when there are more wavelengths used than components to be resolved, multilinear regression can be used to provide the least squares 'fit' of the individual spectrum of each component. The data at the extra wavelength effectively then acts as an internal check upon the accuracy of the proposed algorithm (Cope, 1991).

In most tissues subjected to NIRS, haemoglobin is by far the dominating absorbing substance compared to cytochrome oxidase. Typical in-vivo concentrations are 84 μM for haemoglobin, and 1-5 μM for the cytochrome enzymes. The near infrared spectrum of blood is taken to arise wholly from oxy- and deoxy-haemoglobin, with only minor contributions coming from myoglobin (Mancini *et al.*, 1994). Carboxy- and sulf-haemoglobin, and haemiglobin are only present in minor amounts, and comprise less than 1% of the haemoglobin signal in normal subjects (Cope, 1991).

In the case of oxy- and deoxy-haemoglobin, the total volume of these substances (HbO_2 + Hb - total blood volume) can be expected to change over the course of a study. The concentration of the respiratory enzymes can be assumed not to change over the same period. Any change observed in the cytochrome oxidase

oxidation state will not be as a result of a change in enzyme concentration, but merely in its redox state (i.e. $Cyt_{oxidised} + Cyt_{reduced}$ is constant).

However, the much lower concentration of cytochrome oxidase makes in-vivo monitoring of CtOx a far more complex problem than the monitoring of haemoglobin. To verify that the signal ascribed to be oxidised cytochrome oxidase was accurate, NIRS studies have been carried out on animals in which the blood was replaced with a fluorocarbon substitute (Wray *et al.*, 1988; Cope, 1991). Obtaining spectra from such preparations perfused with 100% O_2 and 100% N_2 (Wray *et al.*, 1988), contributed to devising the algorithm for monitoring the redox state of cytochrome oxidase in normal blood-perfused tissues. The algorithm for cytochrome oxidase has since been tested in animal studies in which the respiratory chain was fully reduced by the addition of cyanide. The partial pressure of inspired oxygen was then reduced to zero, to cause large decreases in HbO_2 and increases in Hb. This resulted in only minor changes in the cytochrome oxidase signal, and seemed to suggest that any effect on the cytochrome oxidase redox state signal from the haemoglobin signal was likely to be small (Cooper *et al.*, 1994; Cooper *et al.*, 1996). The algorithm developed by Cope (1991) used in the NIRO-500 therefore seemed robust to large changes in haemoglobin concentrations, suggesting that accurate values for changes in HbO_2, Hb, and CtOx concentrations could be obtained in a clinical environment.

OBJECT OF THE RESEARCH

Lactic acid was first observed to increase with exercise intensity by Hill & Lupton early this century. Since then, opinion has been divided as to the actual cause of lactate production. Early evidence suggested that as exercise intensity increased, oxygen availability in the muscle fell. This continued up to a point where it was insufficient to maintain aerobic energy production and anaerobic metabolism had to contribute, generating lactic acid. More recent evidence however, seems to show that oxygen never falls to limiting levels in muscle and that lactic acid may in fact be a useful metabolic intermediate.

The aim of this thesis is then to look at lactate production and the role of oxygen in muscle metabolism.

Each of the following chapters specifically tests different hypotheses.

Chapter 3 - Blood Flow and Lactic Acid Production in a Small Muscle Group, exercising both alone, and simultaneously with a Large Muscle Group.

Hypotheses:

- that increasing intensities of supine leg cycling will eventually reduce the blood flow to a simultaneously exercising forearm.

- that this blood stealing from the forearm as the leg exercise intensity increases will cause the muscle to become gradually hypoxic, but will only lead to elevated lactate production if the trigger to lactate production is oxygen starvation.

Objectives:

1. to measure blood flow in both the exercising and resting forearms after forearm exercise when exercising alone, and with both legs cycling at up to 90% of their own maximum.
2. to monitor the lactate concentration in both venous blood effluxing the exercising muscles (from the antecubital vein of the forearm) and arterial blood (arterialised venous blood from the earlobe) after each bout of arm-only, or leg+arm exercise.
3. to get an indication of the relative changes in blood flow in the skin of the hand and arm, and in the total forearm blood flow during the different combinations of leg+arm exercise.

Chapter 4 - The Oxidation State of Isometrically Exercising Forearm Muscle.

Hypothesis:

- that oxygen never becomes limiting in the human forearm muscle and the oxidation state of the muscle, as determined by Near Infrared Spectroscopy (NIRS), will not become reduced as isometric exercise intensity increases.

Objectives:

1. to perform a series of isometric handgrip contractions at fixed proportions of maximum voluntary contraction (MVC), and to monitor the oxidation state of the exercising forearm muscles using NIRS.
2. to repeat the bouts of isometric handgrip contraction with an occluding cuff inflated around the upper arm, again monitoring the forearm muscle with NIRS.
3. to repeat the most intensive bouts of isometric handgrip contraction whilst breathing hypoxic, and hyperoxic gas mixtures, again monitoring the exercising forearm with NIRS.

Chapter 5 - The Oxidation State of Dynamically Exercising Forearm Muscle.

Hypothesis:

- that exercising muscle will not reach a state of oxygen limitation even during the aerobically demanding conditions of high intensity dynamic exercise.

Objectives:

1. to use NIRS to monitor the oxidation state of dynamically exercising forearm muscle when performing the same series of dynamic forearm flexion exercises as used in Chapter 3.
2. to repeat the experiment 5 times with each of several subjects to get an indication of reproducibility of the NIRS technique.

Chapter 6 - Blood Flow, Deoxyhaemoglobin, Oxyhaemoglobin, and the Oxidation State of Cytochrome Oxidase in a Small Muscle Group Exercising Alone, and Simultaneously with a Large Muscle Group.

Hypothesis:

- that the oxidation state of the exercising forearm muscle will not change during conditions of lactate uptake by the muscle, seen in Chapter 3.

Objectives:

1. to repeat the protocol used in Chapter 3, but monitoring the oxidation state of the forearm muscles instead of sampling blood for lactate concentration determination.
2. to repeat the blood flow measurements using the NIRS haemoglobin change under venous occlusion, and to make an extra blood flow measurement while the legs are still cycling, but after the arm has finished.

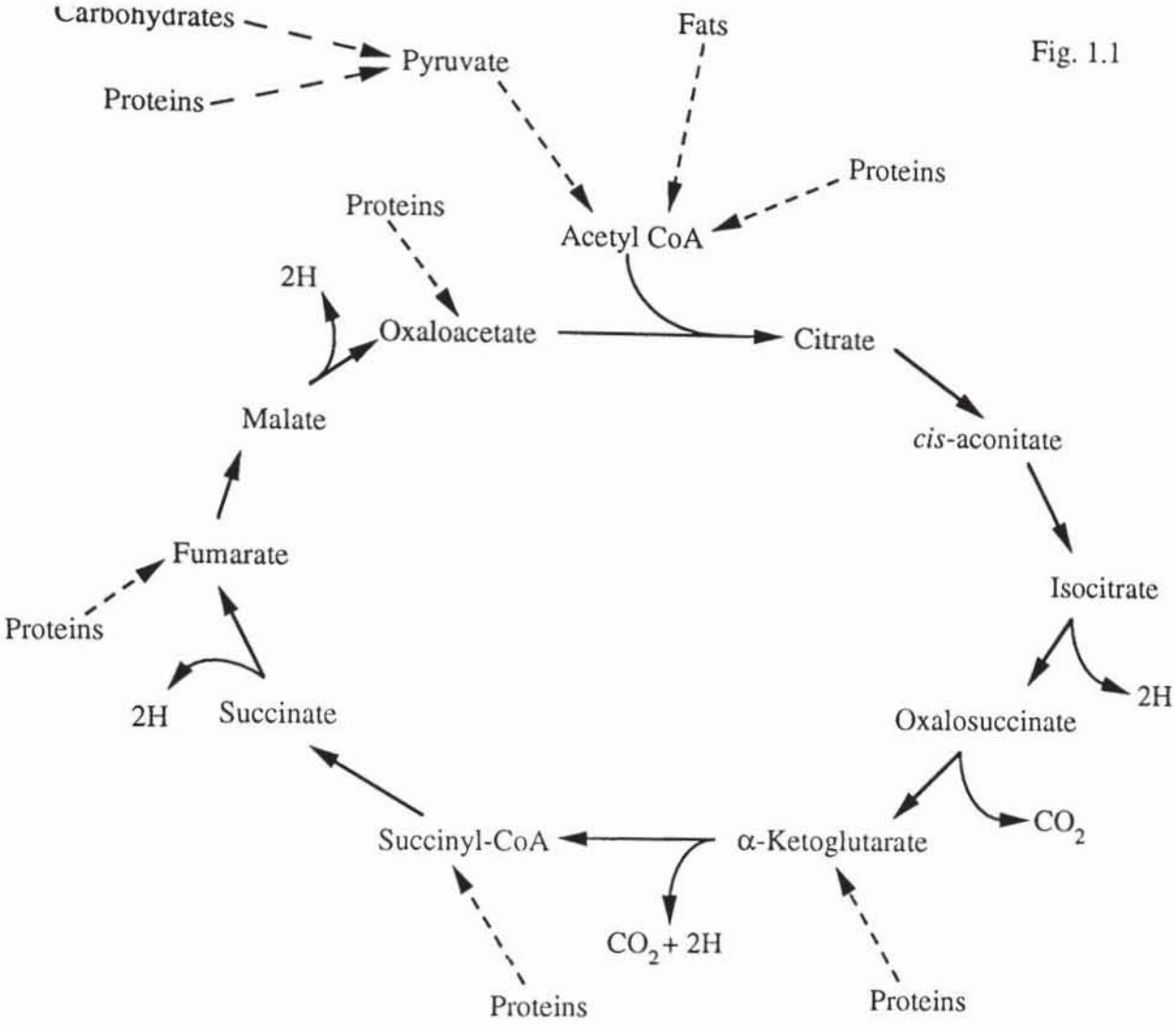
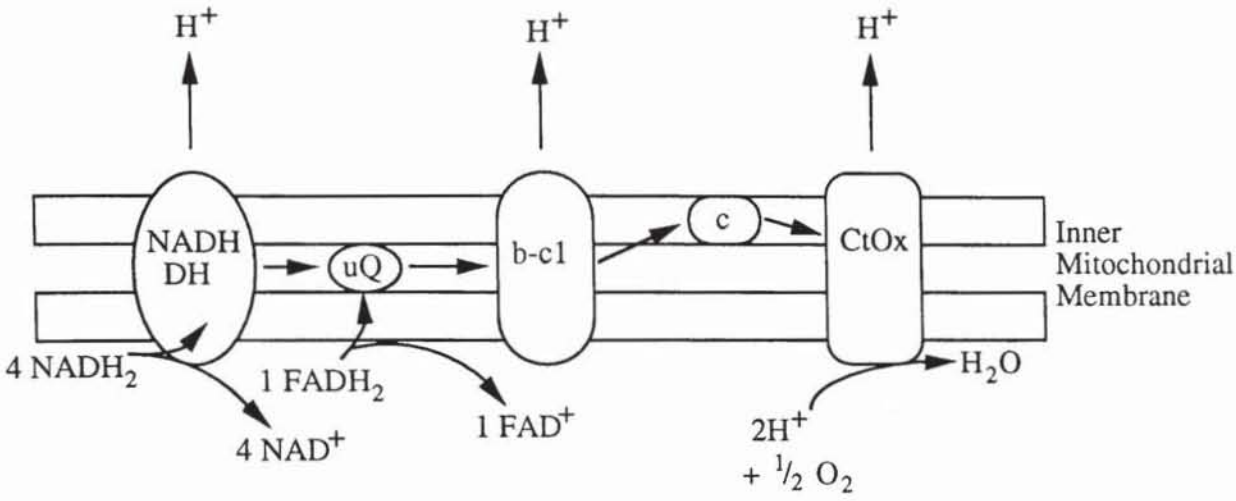


Fig. 1.1: Diagram of the Citric Acid Cycle, showing the points of entry of the various substrates, and location of generation of the products.

Fig. 1.2



Ubiquinone Cytochrome c

NADH Dehydrogenase Complex

Cytochrome b-c1 Complex

Cytochrome Oxidase Complex

Fig. 1.2: Diagram of the Electron Transport Chain, showing the points of entry into the chain of the reducing equivalents carried as $NADH_2$ and $FADH_2$, and order of electron flow.

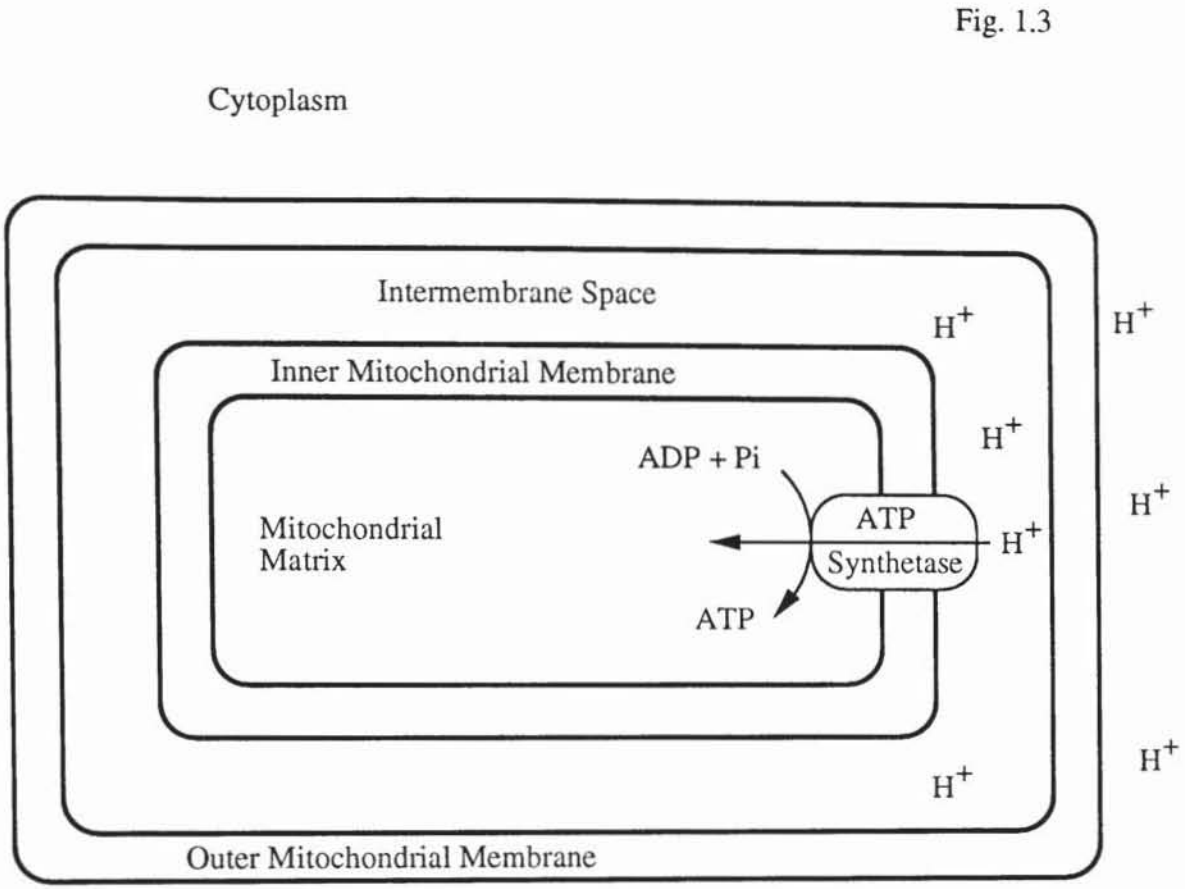


Fig. 1.3: Diagram of a mitochondrion, showing the proton gradient across the mitochondrial membrane, and the action of ATP synthetase.

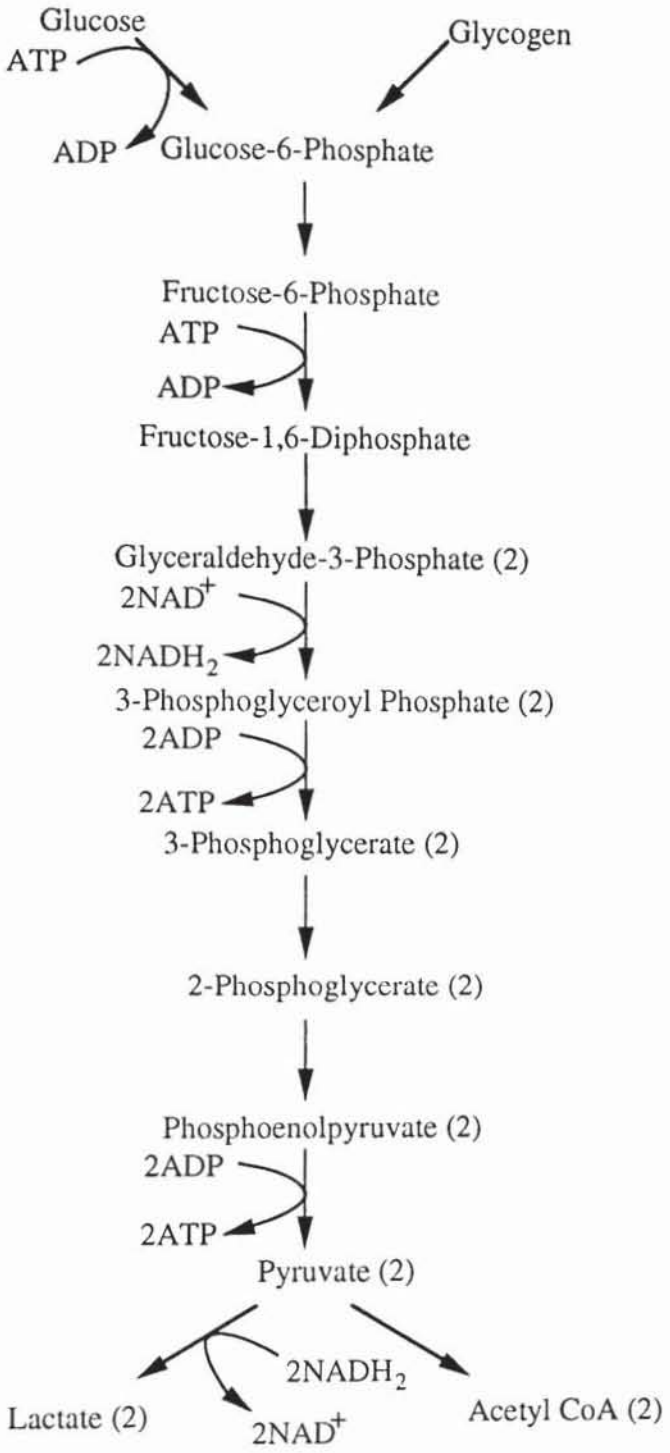


Fig. 1.4: Diagram of the components of the glycolytic chain, and the reactions of glycolysis.

Chapter 2

GENERAL METHODS

This chapter outlines the methods, procedures, and apparatus used for the assessment of physiological variables. Work was carried out either in the Cardiology Exercise Testing Laboratory in the Western Infirmary, Glasgow, or in Glasgow University Exercise Physiology Research Laboratory (BASES accredited). Subsequent chapters outlining specific procedures will make extensive reference to all or part of this chapter.

Subject Preparation and Exercise Mode

Subjects for all studies were recruited from the undergraduate population of the University of Glasgow, and from local competitive sports clubs. They were required to be at least recreationally fit, and partaking in regular exercise. Subjects volunteering for the various studies were first informed of the full requirements of participation, and were made aware of the possible hazards and benefits of the exercise testing procedures. Studies requiring it, namely those involving supine cycling (Chapters 3 and 6), and the study involving breathing different FIO_2 mixtures (part of Chapter 4), were approved by the local Ethics Committee, and subjects agreeing to participate in those studies gave witnessed informed consent (Appendix 1). Subjects did not exercise unless they were in full health, and they were made aware that they could terminate any exercise test at will.

Subjects were asked to arrive at the laboratory in a fully hydrated state, no less than 2 hours after their last, preferably light meal, and to refrain from consuming

tea, coffee, alcohol, or from smoking since their last meal. Before each test, the necessary anthropometric characteristics were recorded. Subject group statistics quoted in each experimental chapter are all of the form mean ± standard error. Where relevant, guidelines laid down by the (then) British Association of Sports Sciences (BASS) in their position statement on the physiological assessment of the elite competitor (1988) were rigidly followed.

All protocols of exercise adopted in this thesis involved some mode of forearm exercise. The dominant, or preferred arm of the subject was always chosen as the active arm, and was denoted as the 'exercising arm.' When used as a non-exercising control, the contralateral arm was denoted as the 'resting arm.' Variables measured from the respective arms were referred to as being 'from the exercising arm,' or 'from the resting arm,' whether measured actually during exercise, or in the rest period following exercise.

WESTERN INFIRMARY LABORATORY

Exercise testing took place in a well ventilated laboratory within the Cardiology Division of Glasgow Western Infirmary.

Determination of Gas Exchange Variables in the Western Infirmary Laboratory

Use was made of the Hospital's MedGraphics Cardiopulmonary Exercise System, which enabled on-line, breath-by-breath measurements to be taken of respiratory variables (principally $\dot{V}O_2$). The system was calibrated before each test according to the manufacturers instructions, by comparison with a known gas mixture (precisely known concentrations of approximately 12% O_2, 5% CO_2, 83% N_2). Ventilatory flow was calibrated using a 3 l syringe. Subjects breathed through a clean, disinfected one way low resistance breathing valve (Hans Rudolf 2600, Kansas City, USA). All analysis was done on dried gas. Previous studies on the validation of the system had found it to be not significantly different to a Douglas bag standard for the principal measured variables ($p = 0.77$; A. Smith, 1994; confirmed by studies collecting the through-flow of expired air using Douglas Bags in series with the MedGraphics system). The laboratory temperature and barometric pressure (determined by thermometer and mercury barometer respectively) were entered before each test took place, to enable the system to accurately apply correction factors for its calculations. All values were corrected to standard temperature and pressure of dry gas (STPD), before calculations of respiratory variables were made.

EXERCISE PHYSIOLOGY RESEARCH LABORATORY

Exercise testing took place in a large, well ventilated laboratory within the Glasgow University Institute of Biomedical and Life Sciences, Division of Neuroscience and Biomedical Systems.

Determination of Gas Exchange Variables in the Exercise Physiology Research Laboratory

While wearing a nose clip, the subjects breathed through a clean, disinfected mouthpiece connected to a one-way low resistance respiratory valve (Hans-Rudolph 2600, Kansas City, USA) attached on the expired air side to a 1 m length of non-kinkable, light weight respiratory tubing (Cranlea, UK). Expired gases were collected into evacuated 200 l Douglas bags (Cranlea, UK) over a timed period. Immediately after collection fractional concentrations of O_2 and CO_2 from each sample were analysed using a paramagnetic O_2 analyser (Servomex 570A) and an infrared CO_2 analyser (P.K. Morgan), and corrections for sample volume made in the final determination of respiratory values. The analysers were calibrated prior to each exercise test using BOC certified gases. Oxygen-free nitrogen was used to calibrate the meters to 0% O_2 and CO_2. A gas mixture in the physiological range (precisely known concentrations of approximately 16% O_2, and 4% CO_2, verified using the Schollander technique), and room air were also used to calibrate and verify respectively the O_2 and CO_2 meter. A dry gas meter

(Parkinson Cowan) calibrated against a Tissot spirometer was used to measure the volume of expired gas. Gas temperature was measured by a probe at the inlet of the volume meter. Barometric pressure was determined with a mercury barometer, and laboratory temperature by a room thermometer. Expired gas volumes were corrected to standard temperature and pressure of dry gas (STPD) using correction factors, before being used to calculate the various respiratory variables.

Determination of Heart Rate

In all exercise testing, heart rate was monitored by short range telemetry using Polar Sport Tester heart rate monitors (Polar Electro OY, Finland). The RR interval electrocardiographic data were detected by a belt worn on the subjects' chest over the xiphoid process, and transmitted to a receiver worn on the wrist. The electrodes of the transmitter belt were dampened prior to use, to aid good contact between the electrodes and the skin. The heart rate data were recorded every 5 seconds throughout exercise. Electronic markers were also inserted into the watch memory, signalling the start/end of exercise, etc.. The stored heart rate data was downloaded onto a personal computer using Polar HR Analysis software (version 3.21A, Polar Electro OY, Finland) for retrospective analysis. Heart rate values for each exercise stage were averaged from the last 30 seconds of data (6 values, 1 recorded every 5 seconds). When the stage was less than 30 seconds

long (e.g. the last 15 seconds of the 4 minute bouts in Chapter 6), all the values were averaged to produce the heart rate value.

Determination of Whole Blood Lactate Concentration

Samples representative of both arterial and venous blood were obtained for blood lactate concentration determination. Arterial samples were obtained as arterialised capillary blood sampled from the earlobe. This has previously been shown to give reliable, representative indications of true arterial blood values when compared to established techniques (Godfrey *et al.*, 1971; Pitkin *et al.*, 1994; findings confirmed by practical experience in our own teaching laboratory). Prior to sampling, the earlobe sampling site was cleaned with a sterile wipe. A sterile Autoclix Lancet (Boehringer Mannheim) was used to manually puncture the earlobe skin. The first drop of blood was wiped away with a tissue and then a 20-25 µl sample was drawn up into an Analox capillary tube (Analox Instruments, London, England) for the purpose of lactate analysis. The tubes were coated with fluoride, lithium heparin, and nitrite, to prevent the blood from clotting and halt any further metabolism in the blood sample which might alter the concentration of the metabolites of interest. Blood samples were thoroughly mixed either manually, or by inversion on a rotating device designed in-house for this purpose, which rotated at 1 Hz. Mixing was continued for 2-3 minutes. Mixing for between 2 and 10 minutes had previously been shown to be adequate to allow the blood to mix

with the tube coating to stabilise the blood, and to give reliable lactate concentrations.

Venous samples were obtained from the forearm antecubital vein in the exercising arm. A cannula was inserted prior to the exercise test, by a medically qualified operator. Following each exercise bout blood was sampled, as detailed in the respective chapters, by withdrawing a sample into a syringe, and then immediately passing 20-25 µl carefully into an Analox fluoride/nitrite/lithium coated capillary tube (Analox Instruments Ltd., London, England) to be mixed as previously described, prior to analysis. The cannula was then flushed with 1 ml of 10 units per ml sterile heparinised saline solution (Heparin Sodium (Mucous) BP in Sodium Chloride Injection BP).

Duplicate 7 µl samples were then analysed using an Analox p-GM7 analyser (in the Western Infirmary Exercise Laboratory) or an Analox GM7 analyser (in the Exercise Physiology Research Laboratory). Prior to use, the analysers were both calibrated using 8 $mmol.l^{-1}$ standards and calibration verified using 5 $mmol.l^{-1}$ standards supplied by the manufacturer. The linearity of the analysers was periodically assessed using dilutions of the 8 $mmol.l^{-1}$ standards supplied, and reproducibility accepted from values within 0.1 $mmol.l^{-1}$ of the known concentration. Lactate concentration was determined from the maximum rate of oxygen consumption caused by the oxidation of L-lactate to pyruvate by the enzyme L-lactate:oxygen oxidoreductase (LOD). Under appropriate conditions, the maximum rate of oxygen consumption is directly related to the lactate

concentration in the sample. Using a 7 µl sample, the reaction is linear to 10 $mmol.l^{-1}$, after which sample size was halved, and the resulting concentration doubled.

$$\text{L-Lactate} + O_2 \xrightarrow{\text{LOD, pH 6.5}} \text{Pyruvate} + H_2O_2$$

All blood samples for lactate determination were analysed in duplicate, the mean of the two values being taken as the true concentration. If the duplicate values differed in concentration by more than 0.2 $mmol.l^{-1}$ lactate, then a third sample was analysed. The mean of the two closest values was then taken as the true lactate concentration. All blood contaminated waste was disposed of in the appropriate manner.

Protocol for the Determination of Maximal Aerobic Power

All tests to determine maximal aerobic power in this study were of a 4 minute discontinuous nature, with 5 minute rest periods between bouts. The protocol was adapted from BASS guidelines (1988) for the assessment of maximal oxygen uptake ($\dot{V}O_{2max}$). Exercise was supine cycling, either on an electronically braked supine cycle ergometer (Siemens Ergomed 740L Couch Ergometer, which adjusts its resistance to maintain a constant load independent of speed of pedal rotation), or on a mechanically braked pendulum type Monark 818 cycle ergometer (Monark AB, Varberg, Sweden) adapted for supine cycling. The Monark

mechanically braked ergometer was regularly checked, and calibrated according to the manufacturers guidelines (Monark AB, Sweden). However, the facilities for verifying the calibration of the Siemens electronically braked ergometer were unfortunately not available.

All subjects carried out a general stretching routine of their choice prior to the test. The subjects were then positioned on the ergometer, and it was adjusted to the subjects' requirements by means of a moveable shoulder rest. Other pre-test preparation was then carried out (see individual experiments), before a 5 minute period of rest after which any pre-exercise measurements were recorded. Subjects all then started the protocol, with a 5 minute warm-up at 60 W, then 4 minute periods of exercise increasing by 30 W per stage to exhaustion, all separated by 5 minute rest periods. Subjects were free to choose any suitable constant pedal cadence which felt comfortable on the Siemens Speed of Rotation Independent ergometer, the rate indicated by an eye-level display, but were asked to maintain 60 rpm on the adapted Monark ergometer. Feedback on the Monark ergometer was via a visual digital display from a computer attached to the ergometer, reinforced by verbal encouragement, and also from an audible metronome if desired. The pendulum position denoting the resistance on the Monark ergometer was regularly inspected, and adjusted as necessary to maintain a constant load.

Respiratory variables were measured during the 4 minute periods of exercise as previously described. Measurement was either continuous throughout the 4 minute exercise period (in the case of the MedGraphics Cardiopulmonary

Exercise System), or in the last minute (in the case of Douglas Bag analysis in the Exercise Physiology Research Laboratory). Mouthpiece and noseclip were applied during exercise periods, but removed during rest to aid subject comfort.

The test continued to exhaustion, signalled by the inability to complete a 4 minute stage, or to start the subsequent stage. The maximum oxygen uptake achieved here was taken as $\dot{V}O_{2max}$, and the maximum power output achieved taken as 100% for use in the subsequent tests.

Follow-up Tests

All follow-up tests were carried out with a minimum recovery of 48 hours after the previous maximal test, at the same time of day as the initial test to minimise the effect of diurnal variation. The discontinuous protocol test was adopted so that the physiological values recorded during the test of maximum aerobic power would reflect as closely as possible the subjects' condition in the follow-up tests which used the same pattern of exercise periods separated by 5 minute periods of rest. These tests began with a leg cycling warm-up at 60 W for 5 minutes, followed by 5 minutes of rest. Then the dominant arm performed a series of simple forearm flexion exercises (see below), for 2 minutes at each of three intensities, with a 5 minute rest period between bouts. The subjects were again supine throughout. During this period, various measurements were made as detailed in the specific methods sections of the respective chapters.

The group of three graded forearm exercise bouts was then repeated a further 4 times, superimposed on top of supine cycling at 30%, 60%, 90%, and then 30% again of the subjects' own individual maximum power output. These bouts generally consisted of 4 minutes of cycling, with the forearm exercise superimposed in the latter half of the exercise period. The exact timing is detailed in the respective chapters (Chapters 3 and 6). Five minute periods of rest again separated each exercise bout, in which all the necessary measurements were taken. The final 30% stage was performed to ascertain the effects of the 60% and 90% exercise stages on the parameters measured.

Dynamic Forearm Exercise

Dynamic forearm exercise in this thesis involved simple wrist flexion exercises, lifting a handpiece (153 mm x 68 mm x 17 mm) held in the flat, upturned palm of the dominant hand, in a rhythmic manner once every 2 seconds in time with a metronome if desired (that is, the forearm muscles contracted and relaxed once every 2 seconds, Fig. 2.1). Subjects were constantly encouraged to use their full range of wrist movement, and this was monitored visually. The handpiece was held in the flat palm, secured using the thumb on the upper surface. Forearm exercise was always in bouts of 2 minutes duration. The subjects were instructed to use only their forearm muscles, their hand pivoting at the wrist, and to avoid the use of their biceps with their forearm supported on a flat surface. Three intensities of dynamic forearm exercise were used:

1) the weight of the handpiece alone (100 g), termed '0 kg',

2) with 1 kg attached, suspended from the handpiece, termed '1 kg',

3) with 1.5 kg or 2 kg suspended from the handpiece, termed ' "2" kg'.

It was originally intended to use the weight of the handpiece alone as the first intensity, then 1 kg, and finally 2 kg for all subjects. Preliminary experiments however, proved misleading, pilot subjects apparently having above average forearm strength-endurance capacity. The majority of subjects could in fact only manage 1.5 kg at maximum. The last intensity was always chosen to be the maximum the subject could manage for 2 minutes.

This exercise used the muscles largely contained in the anterior compartment of the forearm. The main muscles involved are (Johnston & Whillis, 1949; Gosling *et al.*, 1993):

Flexor carpi radialis -	origin:	medial epicondyle of humerus.
	insertion:	base of second and third metacarpal bones.
Flexor carpi ulnaris -	origin:	medial epicondyle of humerus, and olecranon process of ulna.
	insertion:	the pisiform, hamate, and fifth metacarpal bones.

Flexor digitorum superficialis -	origin:	medial epicondyle of humerus, medial surface and coronoid process of ulna, and shaft of radius.
	insertion:	by four tendons onto middle phalanges of each finger.
Flexor digitorum profundus -	origin:	shaft of ulna.
	insertion:	by four tendons, one to terminal phalanges of each finger.

Other muscles, such as the brachioradialis, the pronator teres, the pronator quadratus, and the flexor pollicis longus, and some of the muscles in the posterior compartment of the forearm also act as weak flexors of the wrist, and assist in dynamic wrist flexion exercise.

No one muscle was therefore involved in forearm exercise. However, where (such as in the NIR spectroscopic studies) one muscle needed to be specifically identified as involved in wrist flexion (or isometric wrist contraction - see below), the flexor carpi radialis, as the most dominant muscle, was selected. Otherwise, the muscles used will be described as the "forearm muscles".

Isometric Forearm Exercise

Isometric forearm grip exercise was assumed to use principally the same muscles as outlined above for dynamic forearm flexion. When needing a specific muscle therefore, the flexor carpi radialis was again used. Grip strength testing, and isometric handgrip exercise was carried out on a handgrip dynamometer. This was manufactured in house, and consisted of a fixed bar which located in the palm of the hand, and a second bar around which the fingers of the hand gripped (Fig. 2.1). The exercise involved trying to squeeze the two bars together in isometric grip contraction. The second bar was attached to a strain gauge (RS 100 kg Load Cell, stock number 645-805). The output of the load cell was displayed on a digital readout, and also fed into computerised chart recording equipment (a Macintosh LCII computer, running MacLab Chart v. 3.3.5, ADInstruments, Australia).

Near Infrared Spectroscopy (NIRS)

The concentrations of oxidised and reduced haemoglobin, and the oxidation state of cytochrome oxidase were monitored by Near Infrared Spectroscopy (NIRS - NIRO-500 Image Processing and Measuring System, Hamamatsu Photonics KK, Japan). The near infrared signal was carried to and from the spectrophotometer along glass optical fibres with optodes at the distal ends of the fibres. These consisted of a small prism reflecting the near infrared laser light through 90° and

into the tissue being monitored. The optodes were attached parallel to the long axis of the arm, on the volar surface of the forearm over the belly of the flexor carpi radialis muscle, with a fixed centre-centre separation of 4 cm. The first study involving NIR Spectroscopy used small, 8 mm sized optodes, which were held 4 cm apart using a specially designed flexible black rubber holder, attached to the skin by a double sided adhesive pad, into which the optodes fitted. Subsequent studies used larger, 20 mm sized optodes, which were attached to the arm via double sided adhesive tape with holes in, cut 4 cm apart for the NIR light to pass through. The optodes were shielded from excessive external light by a black, light-proof cloth placed over the forearm and optodes. The transmitting optode was always positioned proximal to the receiving optode. The NIR spectrophotometer was switched on at least 30 minutes prior to use, to allow the laser diodes to warm up and reach their optimum working temperature (as recommended in the NIRO-500 Instruction Manual, Hamamatsu Photonics KK, Japan). The NIR signal was recorded at either 1 sample per second, or 2 samples per second, and stored on an IBM compatible 486-25 MHz computer. The signals detected were converted to concentration changes of Hb, HbO_2, and CtOx by an algorithm using the specific extinction coefficients of each chromophore, as described in the Introduction (p. 25), and quoted in Appendix 2.

The optodes were attached in parallel to the long axis of the arm, to counter any possible change in the optode geometry from limb hyperaemia during exercise. In this orientation, the optode position relative to one another was anticipated not to vary. This is supported by the lack of any regular, reproducible change in the

NIRS signals when under venous occlusion. This is discussed further in Chapter 6 (p.244).

Forearm Blood Flow

Several methods were used to determine total blood flow in the forearm. Venous occlusion strain gauge plethysmography was used to measure the blood flow in both the resting and exercising forearms in the work described in Chapter 3. Mercury-in-silastic strain gauges were used (Hokanson, USA) attached to Medasonics Strain Gauge Plethysmographs (California, USA). Venous occlusion was provided by cuffs wrapped around the upper arm and inflated to 50mmHg in 0.3 second by a Rapid Cuff Inflator (E-20, Hokanson, USA), attached to a Rapid Cuff Inflator Air Source (AG-101, Hokanson, USA). Venous occlusion was maintained for 15-20 seconds. Signals from the Plethysmograph were fed into a computer which recorded the strain gauge data, and printed it out in graph form ready for analysis. For every blood flow measurement the strain gauges were attached around the widest part of the forearm, with the cuff proximal enough to avoid any "cuff artefact" (Abramson *et al.*, 1939; Greenfield *et al.*, 1963; Sumner, 1985). Suitable sizes of strain gauge were used for the subject, the strain gauges allowing up to 4 cm stretch. The forearm was held relaxed at approximately heart level by the subjects' side, when he/she was lying in a supine position. Verbal encouragement was given continuously to prevent inadvertent arm movement during measurement.

Prior to each blood flow measurement, a 1% calibration signal was applied to each strain gauge, corresponding to a 1% increase in limb volume. The actual blood flow was then calculated manually by determining the slope of the initial portion of the graph, in divisions per minute, and dividing by the calibration signal, in divisions for a 1% change in limb volume (Sumner, 1985). This produced blood flow in $ml.\%^{-1}.min^{-1}$ (or $ml.100ml^{-1}.min^{-1}$ - Equation 1).

Equation 1:

$$\text{Flow } (ml.100ml^{-1}.min^{-1}) = \frac{\text{Slope } (div.min^{-1})}{\text{Calibration } (div.1\% \text{ volume change}^{-1})}$$

In the NIRS experiments, the blood flow was recorded by the rate of increase of the total haemoglobin signal (HbT - the oxyhaemoglobin signal plus the deoxyhaemoglobin signal) under venous occlusion. Occlusion was achieved by the same means as previously described. The blood flow calculations were based on the method of De Blasi *et al.* (1994). The initial linear rate of increase of the HbT signal was calculated by fitting of a regression line to the data points, using Microsoft Excel v.5.0. From the equation of this line, the gradient was determined, giving the rate of inflow of haemoglobin in $\mu mol.l^{-1}.min^{-1}$. Assuming 1 mole haemoglobin has a molecular weight of 64000 g, the amount of haemoglobin inflow into the forearm was calculated in $g.min^{-1}$. Taking the average haemoglobin

concentration of human blood to be 15 $g.dl^{-1}$, the blood flow was calculated, expressed as $ml.100ml^{-1}.min^{-1}$.

e.g.

Under venous occlusion:

Hb increase = 10 $\mu mol.l^{-1}.min^{-1}$.

HbO_2 increase = 35 $\mu mol.l^{-1}.min^{-1}$.

So HbT increase = 45 $\mu mol.l^{-1}$.

45 $\mu mol.l^{-1}$ haemoglobin flowing into the forearm each minute.

Molecular weight haemoglobin = 64000 (1 mole Hb = 64000 g).

45 $\mu mol.l^{-1}.min^{-1}$ = 2.88 $g.min^{-1}$.

2.88 g haemoglobin flowing into the forearm each minute.

Average [Hb] = 15 $g.dl^{-1}$ blood.

2.88 $g.min^{-1}$ = 19.2 $ml.min^{-1}$ blood.

Blood Flow = 19.2 $ml.100ml^{-1}.min^{-1}$.

Skin Blood Flow

Skin blood flow contribution was also determined in a small number of subjects using Laser Doppler Blood Flowmetry (Laser Doppler Blood Flow Monitor MBF3, Moor Instruments Ltd., Devon, England). The flowmeter detects the

doppler shift in laser light (wavelength 810 nm) reflected from red blood cells moving under the probe.

The probe was positioned in appropriate probe-holders (Moor Instruments Ltd.) either on the back of the hand, or on the volar surface of the forearm, on either the resting or exercising arm. The probe-holders were attached by means of double sided adhesive discs attached to the skin blood flow measuring sites of the hand and forearm. The mean red blood cell flux value from the Blood Flow Monitor was recorded (made up from the average speed of the red blood cells, and the total number of cells detected), giving an indication of skin blood flow. Total forearm blood flow was also measured by venous occlusion strain gauge plethysmography, as previously described.

The respective measurements were made in a fixed order, and began immediately on cessation of each exercise bout. They consisted of a 20 second measurement from the hand site, then the probe was moved to the forearm probe holder where a further 20 second measurement recorded. Total forearm blood flow was then recorded, by venous occlusion strain gauge plethysmography for approximately 20 seconds. All skin blood flow data were taken from the last 10 seconds of the 20 second measurement period. This had been shown to be sufficient to allow the signal to stabilise after probe repositioning to within natural variation (18.80% variation over the latter 10 seconds without probe movement, compared to 18.79% variation after repositioning). Two separate testing sessions were required, to enable both the resting and exercising arms to be analysed.

Procedures for Data Analysis, Interpretation, and Presentation

Extensive use of Microsoft Excel v.5.0 was made to analyse the data, and display it in graphical form. Statistical analysis was done using Minitab v.10.1. Curve fitting, and regression line generation was carried out using Excel. Procedure diagrams were drawn using MacDraw II (Claris Corporation). All values quoted in the text are of the form Mean ± Standard Error of the Mean (S.E.M.). All error bars on the graphs are Standard Error Bars.

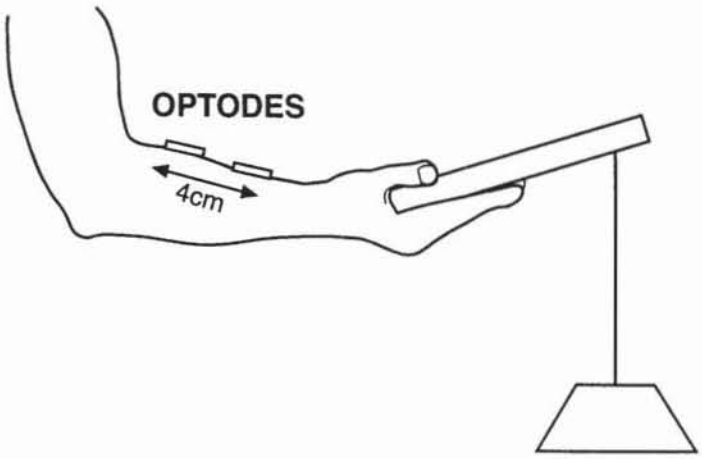
Safety

For all exercise tests, subjects were informed that they were free to terminate exercise if they felt dizzy or nauseous, or at any other time should they so wish. In the case of $\dot{V}O_{2max}$ determination, medically qualified personnel were either present, or on-call and aware of the occurrence of the exercise test. Electrocardiographic activity was also monitored during maximum intensity tests, ready for use in case of any eventualities.

Experimenters handling blood samples wore latex examining gloves, and appropriate laboratory attire at all times. Used sharps and capillary tubes, and all blood contaminated materials (swabs, gloves, tissues etc.) were discarded into sealed BioHazard containers for subsequent incineration. Subjects' skin was cleaned before and after sampling in the case of the earlobe blood samples, and

the cannula flushed with sterile heparinised saline after each forearm blood sample. All mouthpieces and valves were soaked in diluted Milton sterilising fluid (2% sodium hypochlorite, Proctor and Gamble (Health and Beauty Care) Ltd., England) as per manufactures instructions to achieve sterility, and rinsed thoroughly prior to use. The exercise apparatus was thoroughly wiped down and cleaned after each exercise test. The Analox waste fluid was discarded into Cidex (glutaraldehyde, Surgikos Ltd., England) until deemed safe according to the manufacturers' instructions.

DYNAMIC EXERCISE



ISOMETRIC EXERCISE

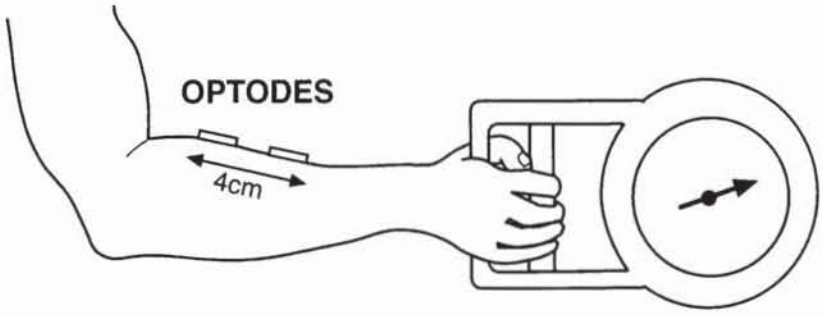


Fig. 2.1: Diagrammatic representation of the actions involved in dynamic and isometric forearm exercise.

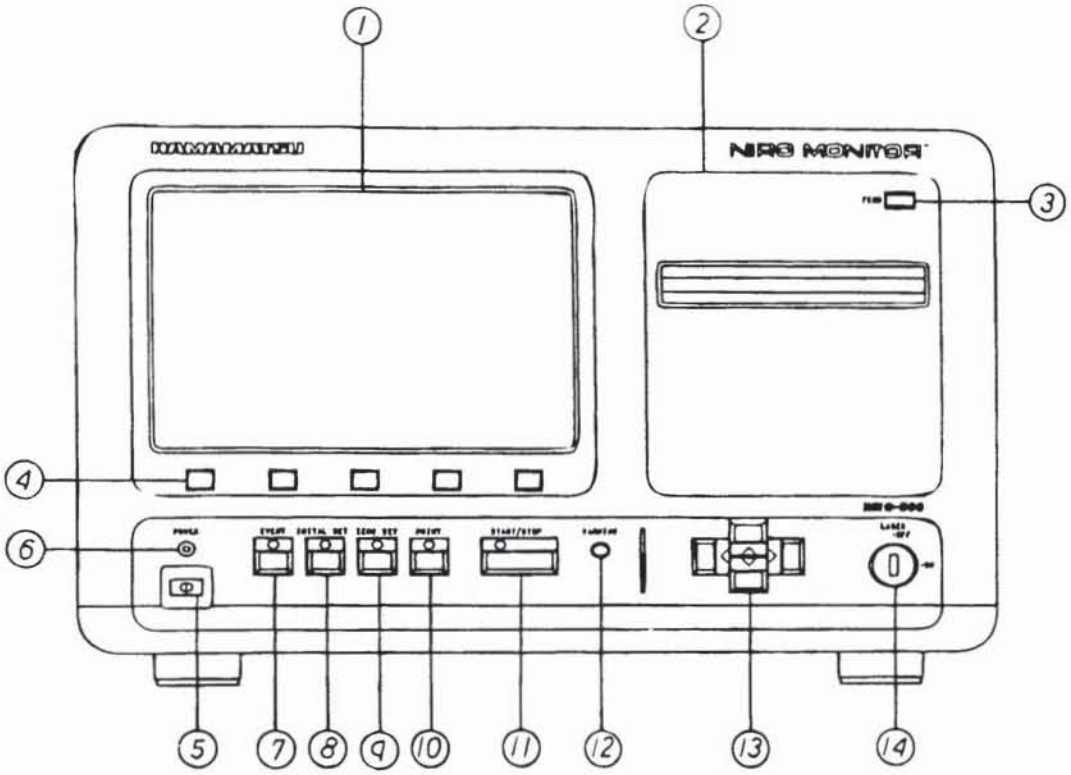


Fig. 2.2: Schematic view of the NIRO 500.

1	Display Panel	8	Initial Set Switch
2	Printer	9	Zero Set Switch
3	Feed Button	10	Print Switch
4	Menu Function Key	11	Print Switch
5	Power Switch	12	Warning Light
6	Power LED Indicator	13	Cursor Arrow Keys
7	Event Switch	14	Laser Key Switch

BLOOD FLOW AND LACTIC ACID PRODUCTION IN A SMALL MUSCLE GROUP EXERCISING BOTH ALONE, AND SIMULTANEOUSLY WITH A LARGE MUSCLE GROUP

INTRODUCTION

This chapter considers the relationship between lactic acid production and blood supply. As noted in the Introduction (p. 21), Saltin (1985) calculates that as little as one third to one half of the muscle mass of an average individual can, when maximally active, require the full cardiac capacity. In whole body exercises involving a greater proportion of muscle mass than the heart can optimally supply with blood, or in situations when a large muscle mass is superimposed upon a small exercising muscle mass, relative vasoconstriction must occur within the exercising muscles, to maintain blood pressure. In this potential "blood stealing" situation, it seems probable that some regions of the muscle must therefore become oxygen deprived. The O_2 dependency of lactate formation has been supported by numerous studies and reviews (e.g. Hill & Lupton, 1923; Wasserman *et al.*, 1986; Katz & Sahlin, 1988). Wasserman & Koike (1992) took Anaerobic Threshold (AT) to represent the point at which some shortfall of O_2 supply begins, and argued that any shortfall in oxygen supply to the working muscles would only be evident at work intensities above the AT. Providing the workrate still remained below the AT workrate, there would be no affect on lactate accumulation from a reduction in blood flow to the exercising muscles. Below AT, work can be performed totally aerobically, and reductions in oxygen delivery would simply reduce the work intensity, or $\dot{V}O_2$, at which AT occurred. Above AT however, the work is (according to the above thinking) being performed partially anaerobically, so any reduction in oxygen delivery would

increase the anaerobic component of the exercise. This would result in elevated net lactate production.

As also noted in the Introduction (p. 10), more recent research has questioned the idea of lactate formation being dependant on the availability of oxygen. In a small isolated muscle, oxygen never becomes limiting, even at $\dot{V}O_{2max}$, yet lactate is produced (Jöbsis & Stainsby, 1968; Connett *et al.*, 1984, 1986; Stainsby *et al.*, 1989). From this it would follow that lactate production can not be related to oxygen delivery, and competition from a large muscle group such as the legs, leading to "stealing" of blood from the exercising arm, will have no effect on the muscle net lactate production.

This study uses the potential "blood stealing" situation created from competition between exercising muscle groups to investigate lactate production in the hope of distinguishing between the oxygen dependant/independent theories. By exercising the forearm muscle of the dominant arm, a small muscle group, at first alone, then simultaneously with both legs cycling at up to 90% of their own maximum, the greater demand for blood from the legs could be expected to challenge the cardiac capacity. At some point this would lead to a reduction in the blood supply to the exercising arm. Large muscle groups, when exercising intensively, may have ischaemic regions within them responsible for lactate production (Spurway, 1992). A small muscle group is likely to be optimally supplied with blood when exercising alone, with the rest of the body at rest, so it is unlikely to have O_2 deprived regions, which the larger muscle masses may have. Increasing the

intensity of bilateral cycling while simultaneously repeating a series of forearm exercises, will eventually surpass the maximum cardiac capacity, and should reduce the blood supply to the forearm, resulting in areas of O_2 deprivation.

Should lactate production be dependent on oxygen supply, and the theory of the Wasserman laboratory be correct, this will result in elevated lactate production from the forearm muscle. Should it be independent of oxygen supply and Jöbsis, Connett, and Stainsby and their respective colleagues be correct, this situation should have no effect on forearm muscle lactate production.

Experiments were designed as follows:

Experiment 1 tests the hypothesis that reducing the blood flow to a small exercising muscle group (by superimposing large muscle group exercise) will increase its lactate production.

Experiment 2 investigates the changes in arm and hand skin blood flow in a similar exercise protocol.

METHODS

Experiments took place in the Western Infirmary Laboratory (laboratory temperature: 24 ± 0.8 °C; barometric pressure: 760.3 ± 2.2 mmHg). All follow-up experiments were carried out at the same time of day to minimise the effects of diurnal variations.

Subjects - Experiment 1

Ten male subjects who were at least recreationally fit and partaking in regular exercise volunteered for this study (age: 23.7 ± 1.0 years; weight: 73.0 ± 2.6 kg; height: 178.4 ± 2.4 cm; aerobic capacity ($\dot{V}O_{2max}$): 50.7 ± 1.7 $ml.kg^{-1}.min^{-1}$).

Subjects - Experiment 2

Three different male subjects volunteered for this study. The subjects were volunteers fitting the same criteria as for Experiment 1 (age: 27.3 ± 3.6 years; weight: 73.7 ± 3.7 kg; height: 178.9 ± 2.6 cm; $\dot{V}O_{2max}$: 51.7 ± 1.9 $ml.kg^{-1}.min^{-1}$). The limited subject group and hence data collection was due to a very small time window for use of the equipment and the laboratory.

SPECIFIC METHODS

The subject was positioned lying supine upon the electrically braked supine cycle ergometer (Siemens Ergomed 740L Couch Ergometer) throughout the experiments. Subjects wore light clothing and sports shoes for all tests.

Experiment 1

For the maximum oxygen consumption/power output tests, the subjects performed a discontinuous protocol to exhaustion, carried out in accordance with BASS guidelines (1988), as described in the General Methods, Chapter 2 (p. 46). Respiratory variables and heart rate were also all recorded as outlined in Chapter 2 (p. 41, 43).

Follow-up tests consisted of the forearm muscle performing simple forearm flexion exercises, either alone, or superimposed on top of both legs cycling (Chapter 2, p. 48). The subject was again supine throughout the test. Forearm blood flow was measured in both the exercising and resting arms by venous occlusion strain-gauge plethysmography (Chapter 2, p. 54). Heart rate, and venous and arterialised venous blood lactate concentrations were all measured as outlined previously (Chapter 2, p. 43, 44).

Following cannulation, subjects lay at rest on the supine cycle ergometer for 5 minutes before resting blood flow measurements were taken simultaneously in the resting and exercising arms. One minute afterwards, resting venous and arterialised venous blood samples were simultaneously taken and analysed for blood lactate concentration (Chapter 2, p. 44). After withdrawal of the blood samples, a second resting blood flow value was taken, 2 minutes after the first blood flow, and 1 minute after the blood samples. The average of the two blood flow measurements was accepted as the value at the time of blood sampling. After these resting measurements, the subject warmed up at the same intensity as in the aerobic capacity test (60 Watts), after which the blood flow measurements and the blood sampling were repeated.

Forearm exercise consisted of simple forearm flexion, as outlined earlier (Chapter 2, p. 49). This exercise was carried out initially at '0 kg' (although the handpiece weighed 100 g) for 2 minutes, after which there was a 5 minute rest period. As quickly as possible after the cessation of exercise (approximately 15 seconds into the rest period, the length of time to take the handpiece from the subject, and calibrate the strain gauges) a blood flow measurement was taken. At 1 minute after the cessation of exercise, blood samples were simultaneously taken from the ear and the arm by 2 operators. All site preparation (such as swabbing of the earlobe puncture site with an alcohol swab) was carried out in the interval between the release of venous occlusion and blood sampling. After the blood had been sampled, a second blood flow measurement was taken 2 minutes after the cessation of exercise. After a total of 5 minutes rest, the exercise was repeated

with the medium weight ('1 kg'), and then after a further 5 minute recovery period repeated again with the heavy weight (' "2" kg'). Blood flow measurements were taken approximately 1 minute before and 1 minute after the start of every pair of ear/arm blood samples thereafter.

The groups of 3 graded forearm exercise bouts were then repeated a further 4 times, superimposed on top of supine cycling at 30%, 60%, 90%, and then 30% again of the subjects' own individual maximum power output, as described earlier (p. 48), and outlined in Fig. 3.1. Exercise bouts consisted of 4 minutes of cycling, with the forearm exercise superimposed in the last 2 minutes of the 4 minute period. The 4 minute bouts were all separated by 5 minute rest periods, when the 2 blood flow measurements, separated by the ear/arm blood samples, were taken. The whole exercise procedure from the insertion of the cannula took 144 minutes.

Experiment 2

The location, method of $\dot{V}O_{2max}$ determination, and manner of testing was the same as in Experiment 1. The skin blood flow from the centre of the dorsal surface of the hand, and volar surface of the forearm, over the belly of the muscle, was recorded in the exercising and resting arms in two identical experiments, separated by at least 48 hours. Subjects were attached to bilateral strain gauges for venous occlusion blood flow monitoring as before. Probe holders for the skin blood flow measurement were attached by means of double sided sticky discs to

the skin blood flow measuring sites of the hand and forearm. The skin blood flow was recorded using a laser doppler blood flow monitor (MBF3, Moor Instruments Ltd., Devon, England), as described in Chapter 2 (p. 56). Heart rate was again recorded continuously by Polar Sport Tester.

A shorter protocol was used than in Experiment 1. Aerobic capacity, and maximum power output was again determined in a discontinuous manner. The next two experimental sessions were identical except for the site of skin blood flow measurement, which was either on the exercising, or resting arm and hand. The subject was positioned supine throughout and the blood flow monitoring equipment attached, before a 5 minute period of rest. Resting skin (hand and arm) and total forearm blood flow measurements were then recorded as described (p. 56 and 54 respectively). The blood flow measurements were repeated in the first minute of the 5 minute rest period after each bout of exercise. The exercise consisted of three 2 minute bouts of arm-only exercise, using the same apparatus and weight sequence as previously (0 kg, 1 kg, "2" kg). Then the 1 kg arm workload was repeated during the 4 leg cycling intensities of 30%, 60%, 90%, and 30% again of maximum. This whole exercise procedure took 72 minutes. At the end of the experiment, to get an indication of the baseline skin blood flow signal, a measurement was taken from the hand and forearm when an arterial occluding cuff was inflated to 240 mmHg around the upper arm.

RESULTS

EXPERIMENT 1 - ARM BLOOD FLOW, BLOOD LACTATE

Terms Used

All values quoted as kg are the arm loads added to the handpiece i.e. '0 kg,' '1 kg,' ' "2" kg.'

All intensities quoted as a percentage refer to the leg exercise intensity level, as percent of maximum (i.e. 0%, 30%, 60%, 90%, 30%(2)).

The arm-only stages are indicated by '0%,' meaning 0% of maximum leg cycling intensity (i.e. legs not exercising); the first stages at 30% of maximum leg cycling intensity are described as '30%,' the stages at 60% and 90% of maximum leg exercise level termed '60%' and '90%,' and the final stages, repeating 30% of leg maximum termed '30%(2).'

Combinations of leg+arm exercise are described as the leg exercise intensity x the arm workload e.g. '30%(2) x "2" kg.'

Blood flow measurements made immediately after exercise are termed 'BF1,' and those made 2 minutes after exercise are termed 'BF2.' Values from the exercising arm have the subscript 'ex' ($BF1_{ex}$, $BF2_{ex}$), and those from the resting arm have the subscript 'rest' ($BF1_{rest}$, $BF2_{rest}$).

Blood lactate sampled from the antecubital vein of the exercising arm is termed Lac_{arm}, and that sampled from the earlobe is termed Lac_{ear}.

Heart rate values relating to the initial 2 minute leg-only period of the 4 minute leg+arm exercise are termed HR_{leg}, and values relating to the arm-only, or the leg+arm periods termed HR_{arm}.

Analysis of Results

Blood flow values were calculated by hand from the gradient of the initial linear portion of the graph as described in the Chapter 2 (p. 54). Careful positioning of the strain gauges and cuffs minimised any cuff artefact appearing from displacement of tissue down under the strain gauge, or out from underneath it. This was confirmed by experiments showing that when the strain gauge and venous occlusion cuff were located as normal, inflating the cuff to normal venous occlusion pressure, while a proximal cuff on the same arm was inflated to 200 mmHg, caused no change in the strain gauge reading.

Statistical Analysis

Analysis of variance (ANOVA) using repeated measures with 2 fixed factors was applied to the data initially, with Bonferroni comparison follow-up tests. Further statistical analysis was done by breaking the data down, and doing separate 2 way ANOVA tests on the data from each individual arm or leg bout (i.e. looking at the effect of leg intensity on the 1 kg arm workload bouts, or the effect of arm

intensity on the 60% leg intensity bouts etc.) again with Bonferroni follow-up comparisons. Simple Student's **t** tests were also used where appropriate. Statistical significance was accepted at the 5% level, and illustrated graphically by an asterisk (*) relating to within each group of leg or arm exercise intensity values

Heart Rate

The HR values were taken both at the end of the 2 minutes of leg-only exercise preceding the leg+arm exercise (HR_{leg}), and at the end of the arm exercise period (either the arm exercising alone, or superimposed on top of the legs exercising, HR_{arm}).

The HR_{leg} values became significantly greater with leg exercise intensity (Fig. 3.2). There was also a significant progressive increase in HR_{leg} with bout over the three 60% and 90% stages. No difference was seen within the three initial 30% bouts. The average 30% value (92.0 ± 2.5 bpm) was significantly lower than the final 30%(2) bouts (average = 110.3 ± 2.5 bpm), which lay mid-way between the values from the 30% and 60% (average = 120.9 ± 3.8 bpm) stages. In the final 30%(2) stages, the first HR_{leg} value (average = 112.3 ± 2.8 bpm) was significantly higher than from the subsequent two bouts (averages: second bout = 109.9 ± 2.4 bpm; third bout = 108.7 ± 2.4 bpm), which were not significantly different from each other.

The HR_{arm} values showed a similar pattern to the HR_{leg} values (Fig. 3.3). HR_{arm} values again increased with leg intensity. Simultaneously there was a progressive increase in HR_{arm} with arm intensity at all leg intensities. However, other than in the 0% arm-only bouts which cannot be compared, this change largely repeats that seen in the HR_{leg} values, particularly at the heavier leg intensities. Some small differences from the leg-only patterns however, were noted:

1) in the initial 30% leg+arm series, the third exercise bout (at the highest arm load) elicited a significantly higher HR than the previous two bouts,
2) there was no significant difference between any of the HR_{arm} values from the final 30%(2) leg+arm series of bouts.

The average HR_{arm} in the three final 30% leg exercise bouts (113.9 ± 3.5 bpm) again fell from the preceding 90% stage to midway between values from the initial 30% (average 96.7 ± 3.3 bpm) and 60% (127.8 ± 3.8 bpm) bouts.

The HR_{arm} values showed a slight increase compared to the HR_{leg} values (Fig. 3.4). The average increase of 6.5 bpm from HR_{leg} (average = 118.8 ± 3.5 bpm at all leg intensities) to HR_{arm} (average = 125.3 ± 4.0 bpm), was significant at a level of $p < 0.05$ (Fig. 3.4).

Blood Flow

The blood flow was measured in both the exercising and resting arms, immediately after exercise, and again after the blood sample had been taken ($BF1_{ex}$, $BF1_{rest}$, $BF2_{ex}$, $BF2_{rest}$).

$BF1_{ex}$ became greater with exercising arm workload (Fig. 3.5). As the leg exercise intensity increased, $BF1_{ex}$ values became less significantly different. After the arm-only bouts of exercise (0%), all $BF1_{ex}$ were significantly different to each other, increasing with arm workload. During the later stages of the study, in the 90% and 30%(2) leg exercise bouts, only the $BF1_{ex}$ values after the most intensive "2" kg arm workloads were significantly higher than those after the easy 0 kg workbouts. The 1 kg values fell in-between, not significantly different from either. $BF1_{ex}$ readings averaged over the final 30%(2) stages (16.0 ± 1.8 $ml.100ml^{-1}.min^{-1}$) were significantly higher than the averages from all the other leg intensity levels (averages: 0% = 11.0 ± 1.4 $ml.100ml^{-1}.min^{-1}$; 30% = 10.7 ± 1.2 $ml.100ml^{-1}.min^{-1}$; 60% = 12.7 ± 1.4 $ml.100ml^{-1}.min^{-1}$; 90% = 12.8 ± 1.7 $ml.100ml^{-1}.min^{-1}$), but the effect was mainly at the 30%(2) x 0 kg bout.

The $BF1_{ex}$ values also show a general increase with leg intensity (Fig. 3.6). This is much the most marked in the 0 kg bouts when $BF1_{ex}$ showed a progressive rise with leg intensity, which continued through to the 30%(2) x 0 kg stage. The higher arm workloads showed less of an effect with increasing leg intensity, $BF1_{ex}$ values not differing significantly from each other until the final 30%(2) stages.

$BF2_{ex}$ (the values taken 2 min after exercise) showed a similar pattern to $BF1_{ex}$, all generally increasing with both arm intensity and leg intensity (Fig. 3.7). The $BF1_{ex}$ values were generally significantly greater than $BF2_{ex}$ (an average of 12.0 ± 1.0 $ml.100ml^{-1}.min^{-1}$ compared to 8.6 ± 0.6 $ml.100ml^{-1}.min^{-1}$), but this was not always the case. After the 90% leg exercise intensity bouts, the blood flow tended to go on increasing during the first 2 minutes after cessation of exercise. The hyperaemic effect was particularly evident after the 90% x "2" kg bouts, though $BF2_{ex}$ (average = 17.64 ± 1.8 $ml.100ml^{-1}.min^{-1}$) is only non-significantly higher than $BF1_{ex}$ (average = 15.8 ± 2.6 $ml.100ml^{-1}.min^{-1}$). After all the lower-intensity leg bouts, $BF2_{ex}$ was significantly lower than $BF1_{ex}$.

In the contralateral, resting arm, $BF1_{rest}$ generally showed no change with the exercising arm intensity (Fig. 3.9). In the three 30%(2) leg exercise bouts however, $BF1_{rest}$ showed a decrease with increasing arm workload. $BF1_{rest}$ did become greater with leg exercise intensity, from the 60% level onwards, and remained elevated in the 30%(2) bouts (Fig. 3.10).

The $BF2_{rest}$ values generally showed a similar pattern to $BF1_{rest}$ (Fig. 3.8). Again there was generally no significant effect due to the exercising arm workload. After the most intensive bout of 90% x "2" kg, $BF2_{rest}$ again actually became slightly higher than $BF1_{rest}$ (11.0 ± 1.7 $ml.100ml^{-1}.min^{-1}$ compared to 7.5 ± 1.0 $ml.100ml^{-1}.min^{-1}$). This is consistent with the hyperaemic effect seen in the exercising arm

over the same comparison (Fig. 3.7). As in the $BF2_{ex}$ values, the blood flows from the final 30%(2) bouts fell from the values after the 90% leg exercise bouts.

Muscle Blood Flow

By subtracting the resting arm blood flow from the exercising arm blood flow, an indication of the changes in muscle blood flow due to arm x leg exercise was obtained ($BF1_{muscle}$, $BF2_{muscle}$). The $BF1_{muscle}$ increased significantly with exercising arm workload (Fig. 3.11), but tended to drop with increasing leg intensity (Fig. 3.12). There was no difference between the values from the 0 kg arm workloads. In the 1 kg and "2" kg bouts of arm exercise, $BF1_{muscle}$ following the 90% stages was less than after the other leg intensity stages. The 90% x 1 kg reading was significantly less than that after the 30% x 1 kg stage (average 90% x 1 kg = 4.9 ± 1.4 $ml.100ml^{-1}.min^{-1}$, compared to 30% x 1 kg = 9.6 ± 1.2 $ml.100ml^{-1}.min^{-1}$), and the value after the 90% x "2" kg stage significantly less than after the 0% x "2" kg stage (average 90% x "2" kg = 8.3 ± 2.0 $ml.100ml^{-1}.min^{-1}$, compared to 0% x "2" kg = 13.0 ± 1.5 $ml.100ml^{-1}.min^{-1}$).

The $BF2_{muscle}$ was lower than all the $BF1_{muscle}$ values, but they both showed generally the same pattern of change (Fig. 3.13). The muscle blood flow tended to remain elevated in the second blood flow measurement from the 90% x "2" kg bout, 2 minutes after exercise. This is again consistent with the hyperaemic effect

seen in the individual blood flow values from the exercising and resting arms (Figs. 3.7, 3.8).

Blood Lactate

Lactate concentration was determined in blood sampled from both the antecubital vein of the exercising forearm (Lac_{arm}), and from the earlobe (Lac_{ear}).

Lac_{arm} increased significantly with forearm workload (Fig. 3.14), and with leg intensity from the 60% leg intensity bouts (Fig. 3.15). The 90% bouts give higher Lac_{arm} at all arm workloads compared to the other leg exercise levels, peaking at 7.2 ± 0.7 mmol.l^{-1} in the 90% x "2" kg stage. The 90% leg exercise intensity bout also seemed to have a carry-over effect on the first 30%(2) bout (Fig. 3.14), which was significantly higher (average = 4.2 ± 0.7 mmol.l^{-1}) than the following two Lac_{arm} values (2.9 ± 0.6 mmol.l^{-1} after the 30%(2) x 1 kg bouts, and 2.7 ± 0.4 mmol.l^{-1} after the 30%(2) x "2" kg bouts). The 30%(2) x "2" kg value was not significantly different to the 30% x "2" kg value (2.4 ± 0.1 mmol.l^{-1}).

Lac_{ear} showed a broadly similar pattern (Fig. 3.16). The arm workload however did not have such a large effect. There was a marked increase in Lac_{ear} with leg exercise intensity after the 60%, 90%, and final 30%(2) bouts, peaking at an average concentration of 7.5 ± 0.7 mmol.l^{-1} after 90% x "2" kg. After the 30%(2) stages, there was a decreasing Lac_{ear} concentration with increasing arm workload.

similar to the Lac_{arm} results. The arterial (earlobe) blood lactate concentration decreased significantly from 4.7 ± 0.8 $mmol.l^{-1}$ after the 30%(2) x 0 kg bout, to 1.7 ± 0.3 $mmol.l^{-1}$ after the 30%(2) x "2" kg bout.

Veno-Arterial Lactate Difference

Subtracting the arterial (earlobe) blood lactate concentration from the venous (forearm) value, an estimate of net lactate gradient across the exercising forearm muscles could be obtained (Lac_{v-a} - Fig. 3.17). This in turn, represents a first indicator of lactate flux from or into the forearm musculature. After the arm-only exercise, and the easiest leg exercise intensity, there was a large net lactate outflow from the forearm (Lac_{v-a} was positive). As the leg exercise intensity increased, and the arterial lactate concentration rose (Fig. 3.16), the Lac_{v-a} fell until after the most intensive bout of leg exercise, at 90% of maximum. Here, net uptake of lactate by the exercising forearm was significant at the two easier arm exercise intensities (0 kg and 1 kg, $p = 0.004$ and $p = 0.049$ respectively), and just non-significant in the "2" kg bout ($p = 0.17$). As the arterial lactate level decreased after the final 30%(2) bouts, the exercising forearm gradually reverted to net lactate outflow.

If the venoarterial lactate difference is then combined with the muscle blood flow at the time of blood sampling (the average of $BF1_{muscle}$ and $BF2_{muscle}$), a more quantitative indication of the rate of lactic acid output from the exercising forearm

can be obtained (Lac_{output} is the venoarterial lactate difference in $mmol.ml^{-1}$ multiplied by average of two muscle blood flow values in $ml.100ml^{-1}.min^{-1}$, the answer expressed as $\mu mol.100ml^{-1}.min^{-1}$ - Fig. 3.18). The Lac_{output} showed a similar response to Lac_{v-a} (Fig. 3.17), becoming significantly greater with forearm workload after the 0% and 30% stages. These two stages were insignificantly different from each other overall. As leg exercise intensity increased, Lac_{output} then fell. After the hardest workloads at 90% leg intensity, the average Lac_{ear} was always over 6 $mmol.l^{-1}$ (Fig. 3.16). Here, a significant net uptake of lactate occurred (Lac_{output} became negative) after the 0 kg arm workloads ($p = 0.017$). Net uptake was however not significant in the 1 kg, and "2" kg bouts ($p = 0.21$ and $p = 0.32$ respectively). After the 30%(2) stages, Lac_{output} gradually reverted to positive values again as the arterial whole body lactate levels fell over the three arm workloads.

EXPERIMENT 2 - SKIN BLOOD FLOW

Possibilities of statistical analysis were limited due to the small subject group. This experiment followed changes in blood flow in the arm with an estimate of the relative contribution from the skin of the hand and forearm.

Terms Used

Blood flow values from the exercising arm are termed $BF_{ex\text{-}total}$, and values from the resting arm termed $BF_{rest\text{-}total}$. Blood flow values from the respective skin sampling sites are termed $BF_{ex\text{-}hand}$ and $BF_{ex\text{-}arm}$ from the exercising arm, and $BF_{rest\text{-}hand}$ and $BF_{rest\text{-}arm}$ from the resting arm. Subjects were required to perform two identical experiments on separate visits, one visit measuring the exercising arm blood flows, and the other visit measuring the resting arm blood flows.

Heart Rate

HR values (all equivalent to HR_{arm} values) followed the same pattern as seen in Experiment 1. There was little change between the two visits to measure the skin blood flow in the exercising arm (Fig. 3.19), and the resting arm (Fig. 3.20). In both visits, there was a slight increase with increasing arm workload in the arm-only bouts. There was a much greater progressive increase with increasing leg

intensity, rising to 90%, then falling back to mid-way between the 30% and 60% values in the 30%(2) bout.

Blood Flow

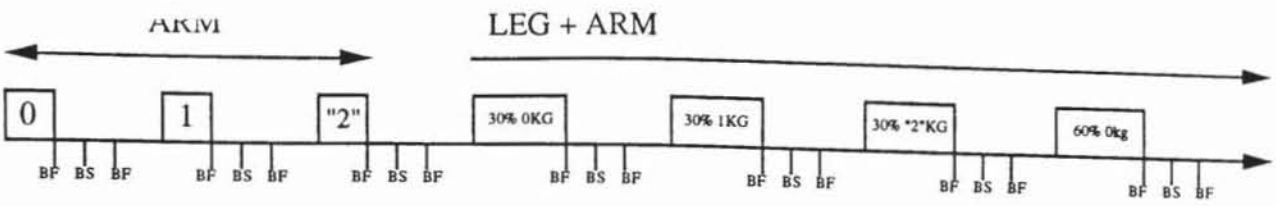
Skin blood flow, measured by Laser Doppler Flowmetry, produces measurements in mV. This means the values from skin blood flow will not be directly comparable to values of total arm blood flow (measured in $ml.100ml^{-1}.min^{-1}$), and only trends of change will be evident.

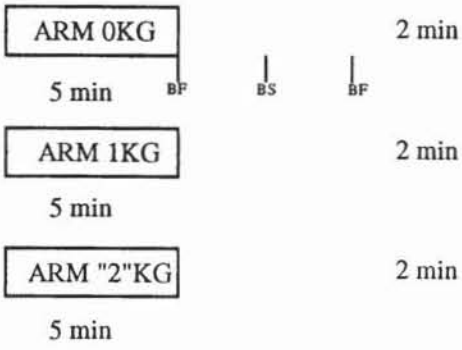
Similar to Experiment 1, $BF_{ex\text{-}total}$ increased from rest with arm workload after the arm-only exercises (Fig. 3.19), but there was no corresponding change in $BF_{rest\text{-}total}$ (Fig. 3.20). There was, in fact, a slight drop in the resting arm blood flow after the "2" kg arm-only bout. There was no change in any of the skin blood flow values from either the exercising or resting arms after arm-only exercise.

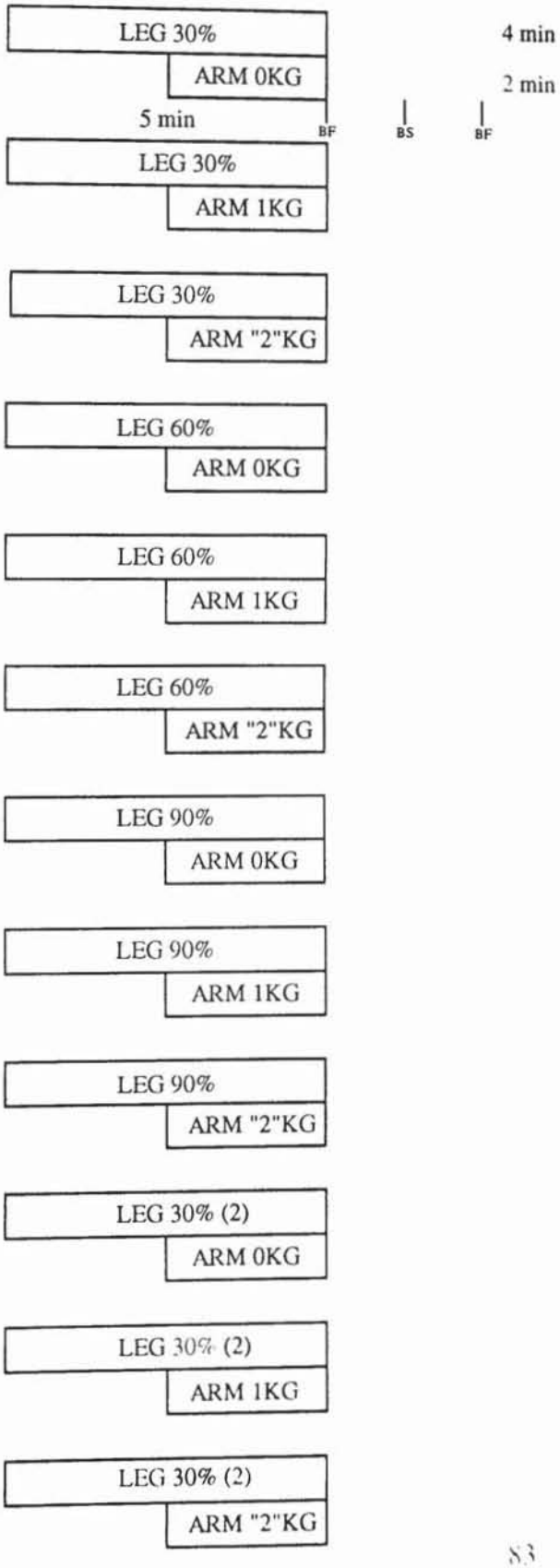
After the leg+arm exercise, there was no change in the resting arm blood flow values ($BF_{rest\text{-}total}$, $BF_{rest\text{-}hand}$, or $BF_{rest\text{-}arm}$) in the first exercise bout of 30% x 1 kg. In the exercising arm, $BF_{ex\text{-}total}$ fell slightly, but as in the resting arm, $BF_{ex\text{-}hand}$, and $BF_{ex\text{-}arm}$ did not change. As the leg intensity increased, the $BF_{ex\text{-}total}$ began to increase again, and the skin blood flow in the exercising and resting arm and hand increased markedly. $BF_{ex\text{-}total}$ dropped slightly after the final 30%(2) bout from the

previous 90% bout, $BF_{rest\text{-}total}$ remained unchanged, but the skin blood flows in both arms continued to increase.

The skin blood flow in the exercising and resting arms and hands tended to change by similar amounts (Fig. 3.21). $BF_{ex\text{-}hand}$ and $BF_{rest\text{-}hand}$ were always greater than $BF_{ex\text{-}arm}$, and $BF_{rest\text{-}arm}$, doubtless due to a greater number of arteriovenous anastomoses and greater number of skin capillaries in the hand (Abramson & Ferris, 1939). Both became equal and close to zero in the resting and exercising arms under total blood flow occlusion from brachial cuffs around both upper arms inflated to 240 mmHg at the end of the experiment.







BF - Bilateral Forearm Blood Flow Measurement

BS - Blood Sample from Earlobe and Forearm

Fig. 3.1
Protocol used when superimposing arm exercise upon leg exercise, when measuring blood lactate (Chapter 3).

Fig. 3.2

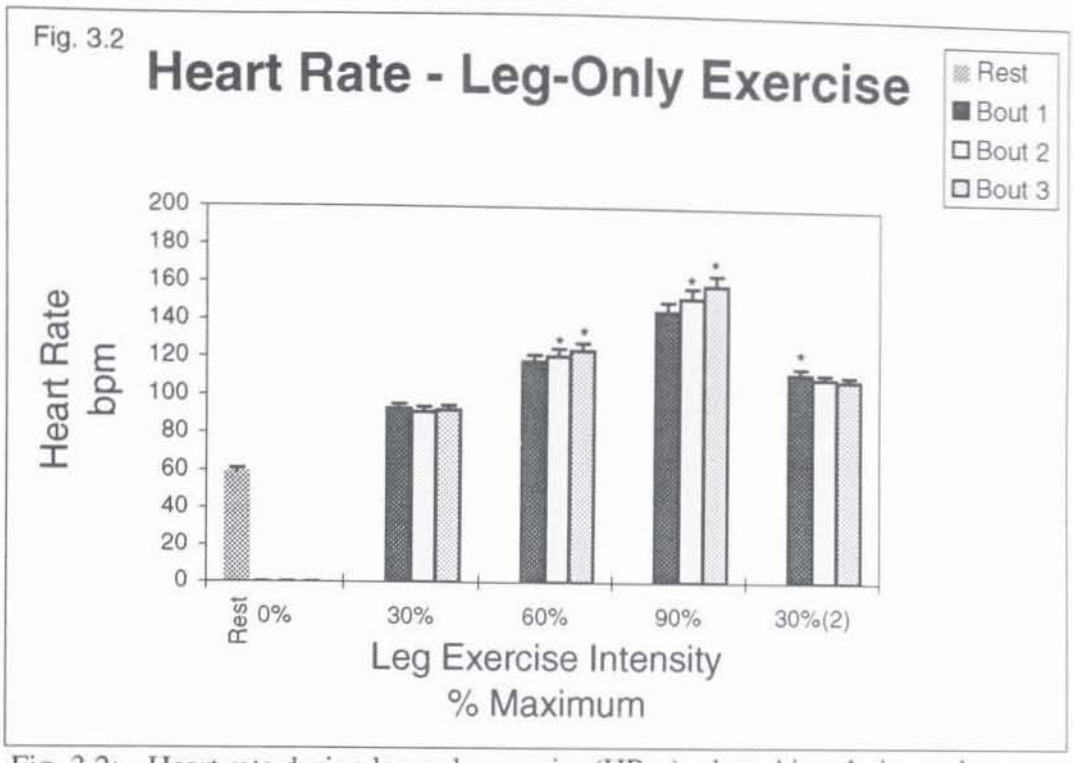


Fig. 3.2: Heart rate during leg-only exercise (HR_{leg}), plotted in relation to leg exercise intensity.

Fig. 3.3

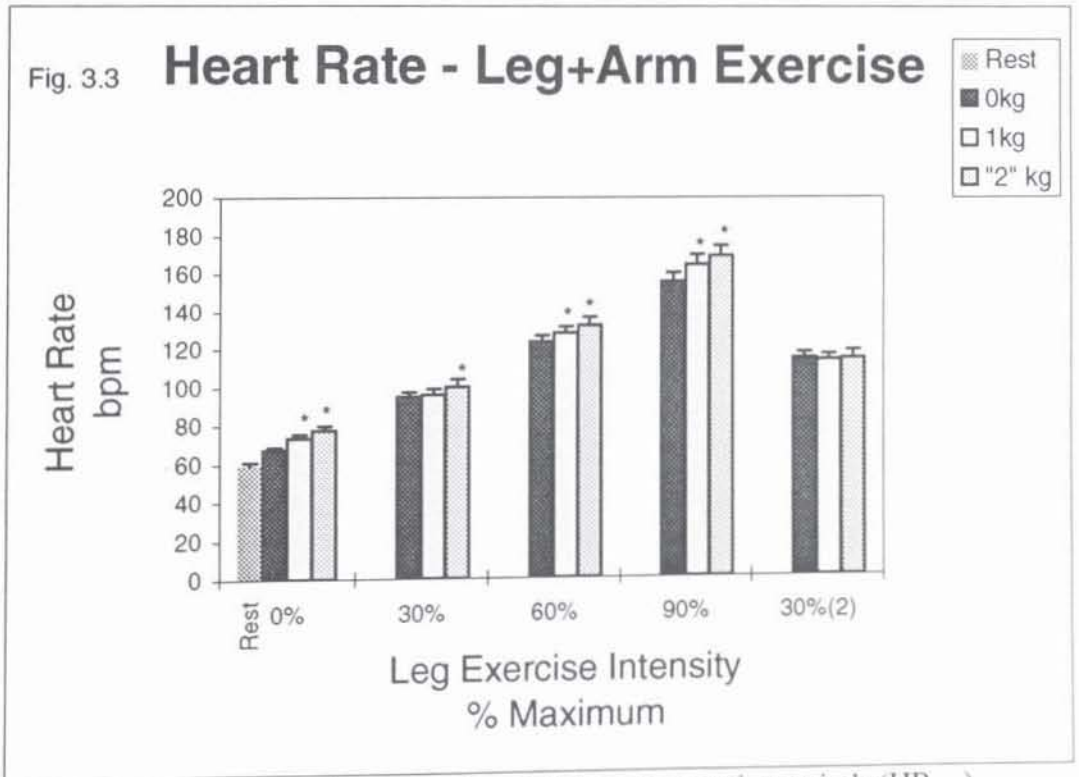


Fig. 3.3: Heart rate during arm-only, and leg+arm exercise periods (HR_{arm}), plotted in relation to leg exercise intensity.

Fig. 3.4

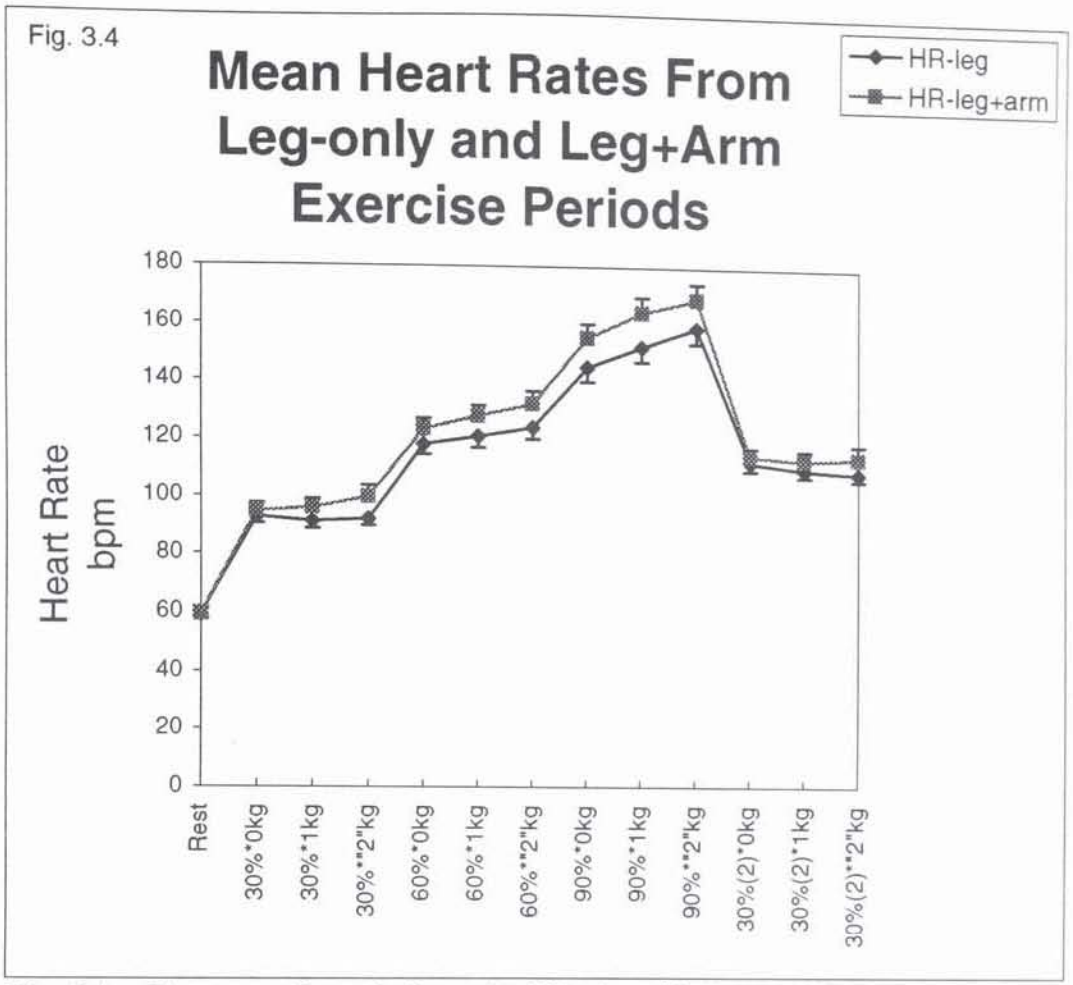


Fig. 3.4: Heart rates from the leg-only (HR_{leg}), and leg+arm (HR_{arm}) exercise bouts, plotted in relation to exercise bout.

Fig. 3.5

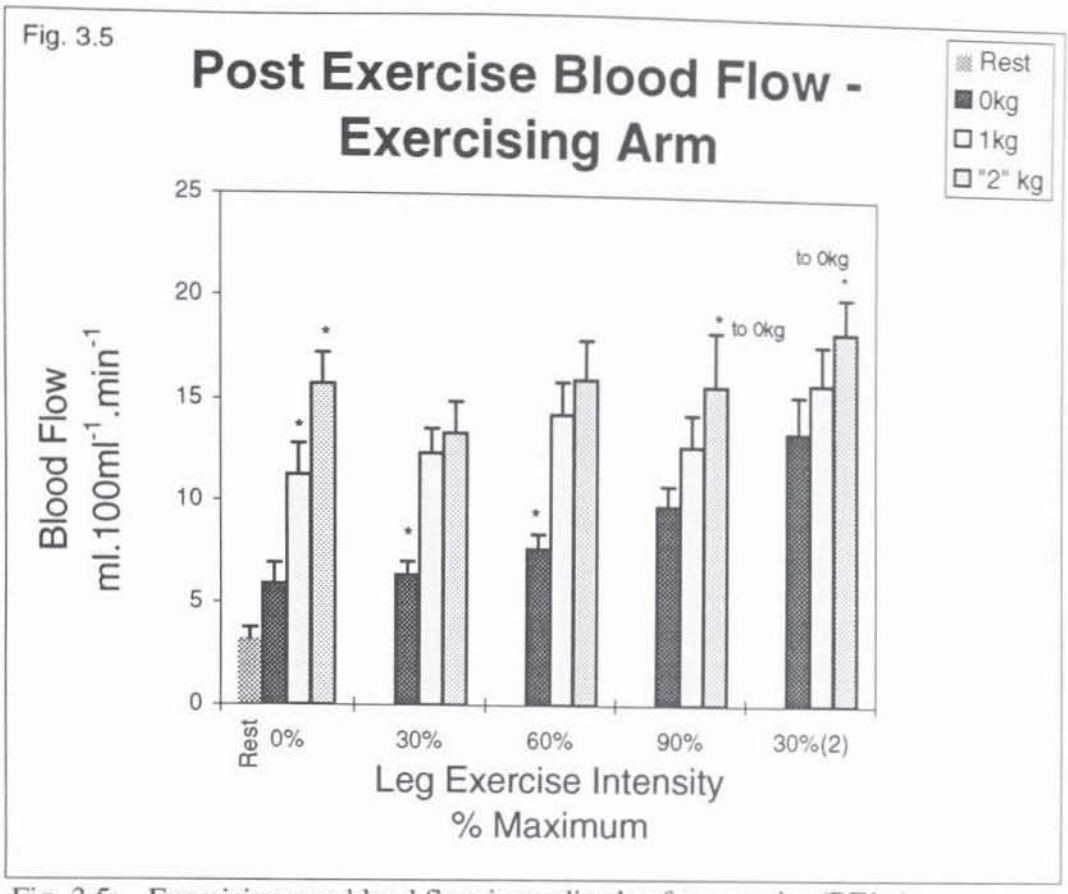


Fig. 3.5: Exercising arm blood flow immediately after exercise ($BF1_{ex}$), plotted in relation to leg exercise intensity.

Fig. 3.6

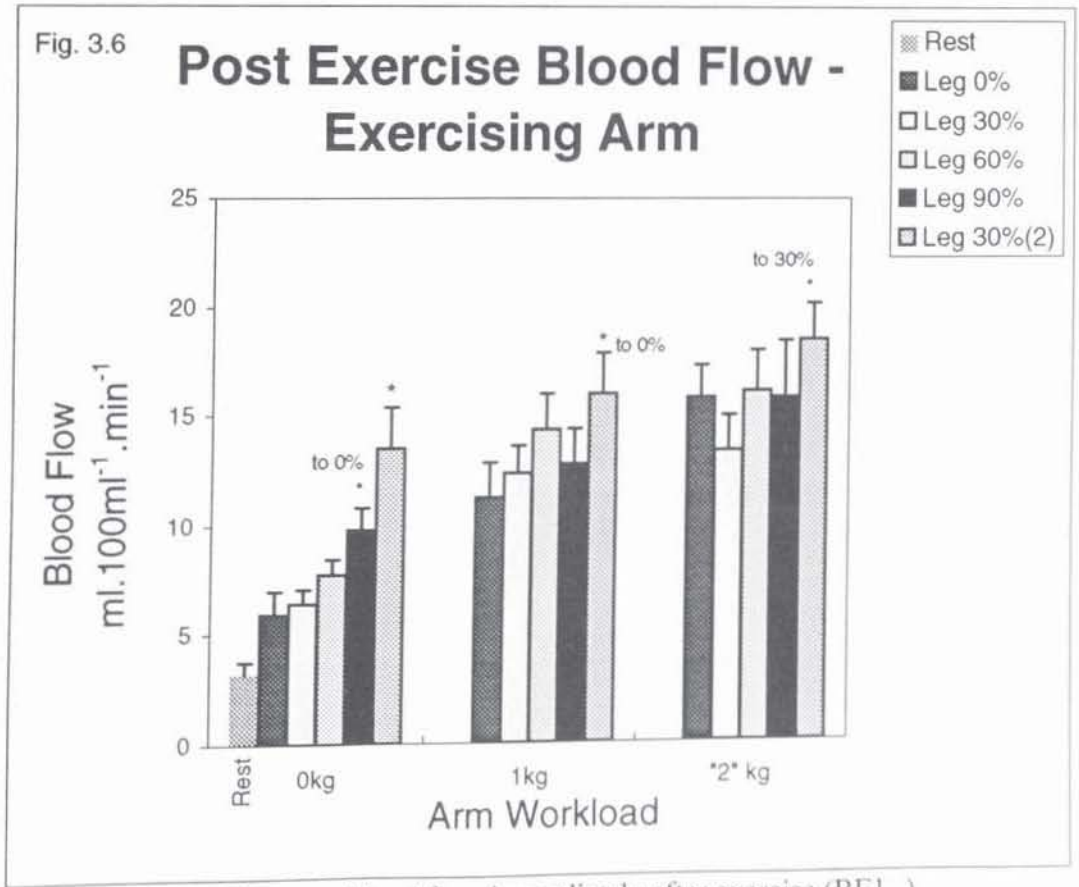


Fig. 3.6: Exercising arm blood flow immediately after exercise ($BF1_{ex}$), plotted in relation to arm workload.

Fig. 3.7

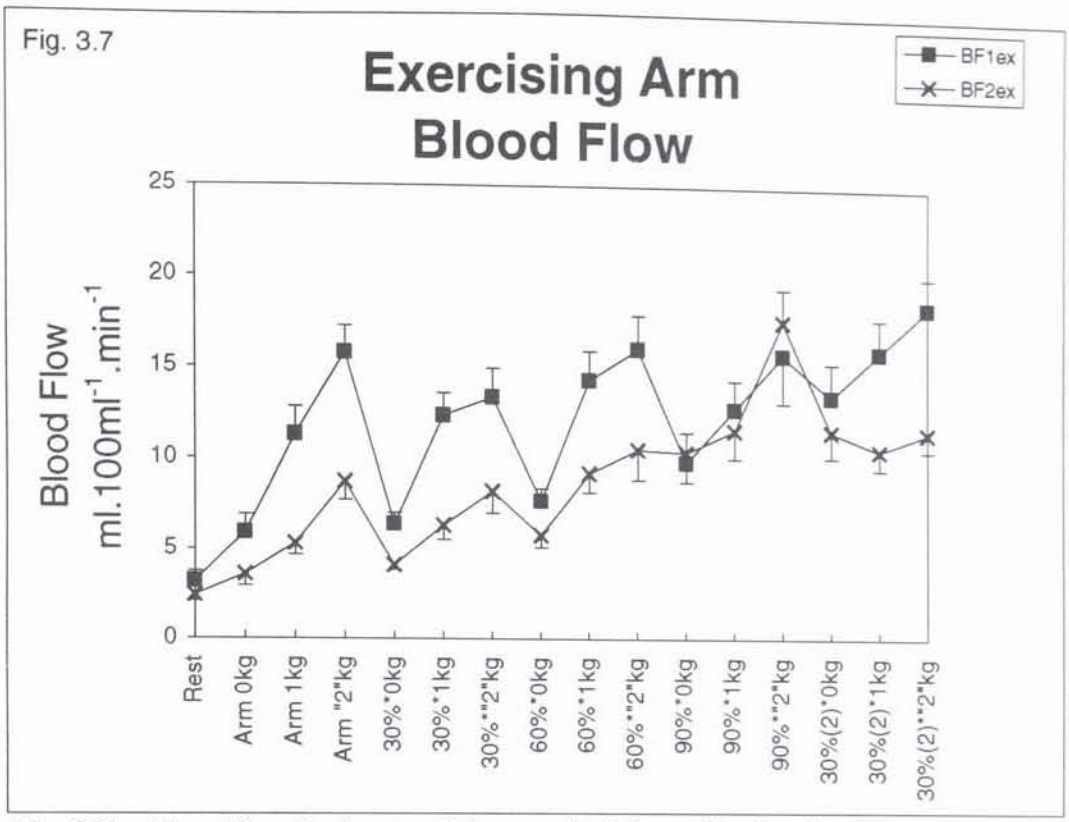


Fig. 3.7: Blood flow in the exercising arm both immediately after exercise ($BF1_{ex}$), and 2 minutes after exercise ($BF2_{ex}$), plotted in relation to exercise bout.

Fig. 3.8

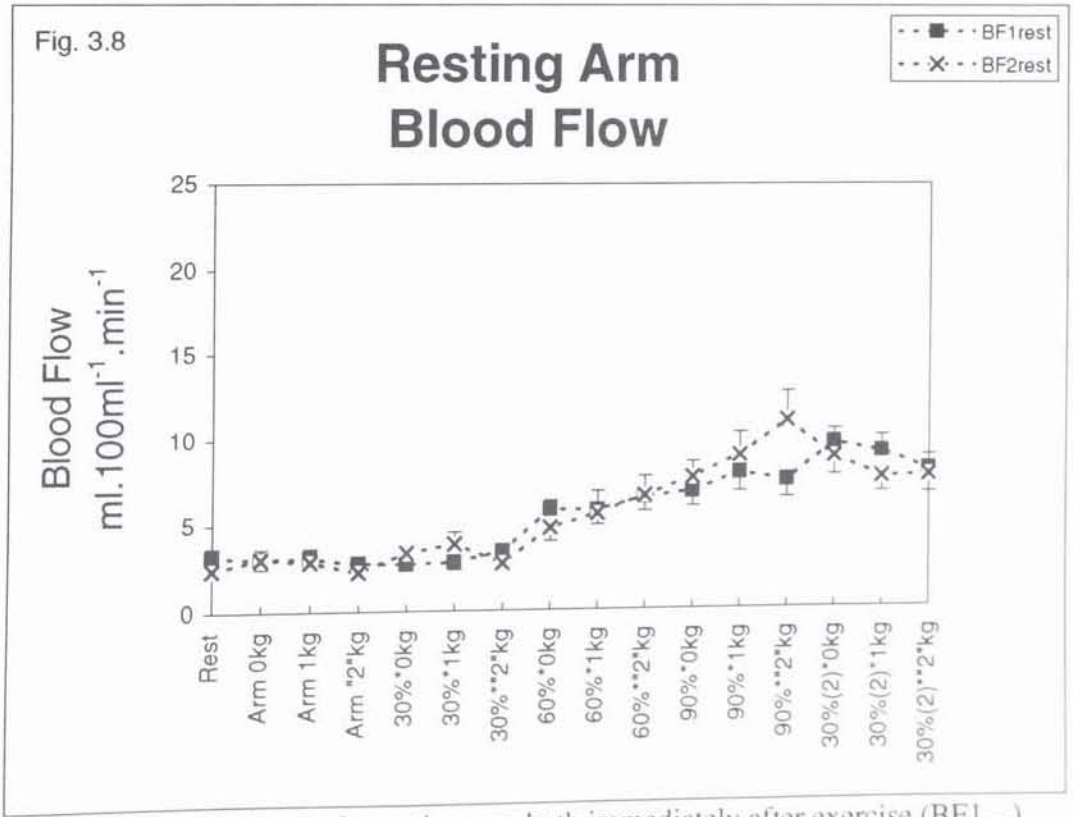


Fig. 3.8: Blood flow in the resting arm both immediately after exercise ($BF1_{rest}$), and 2 minutes after exercise ($BF2_{rest}$), plotted in relation to exercise bout.

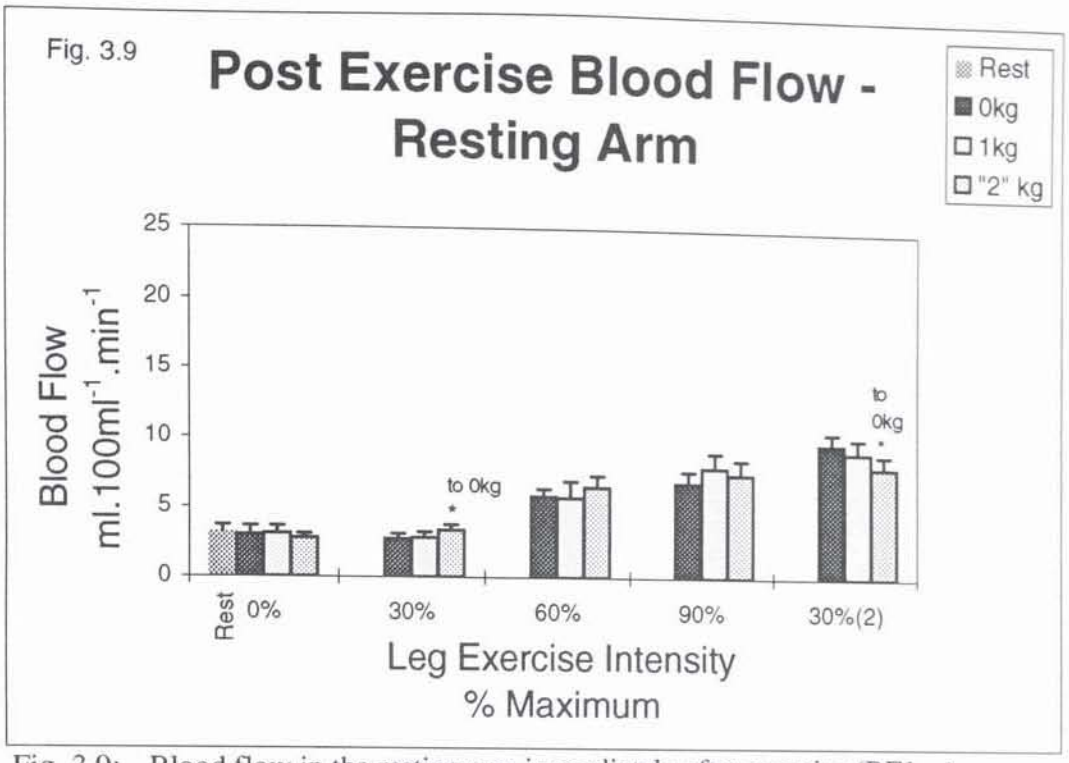


Fig. 3.9: Blood flow in the resting arm immediately after exercise ($BF1_{rest}$), plotted in relation to leg exercise intensity.

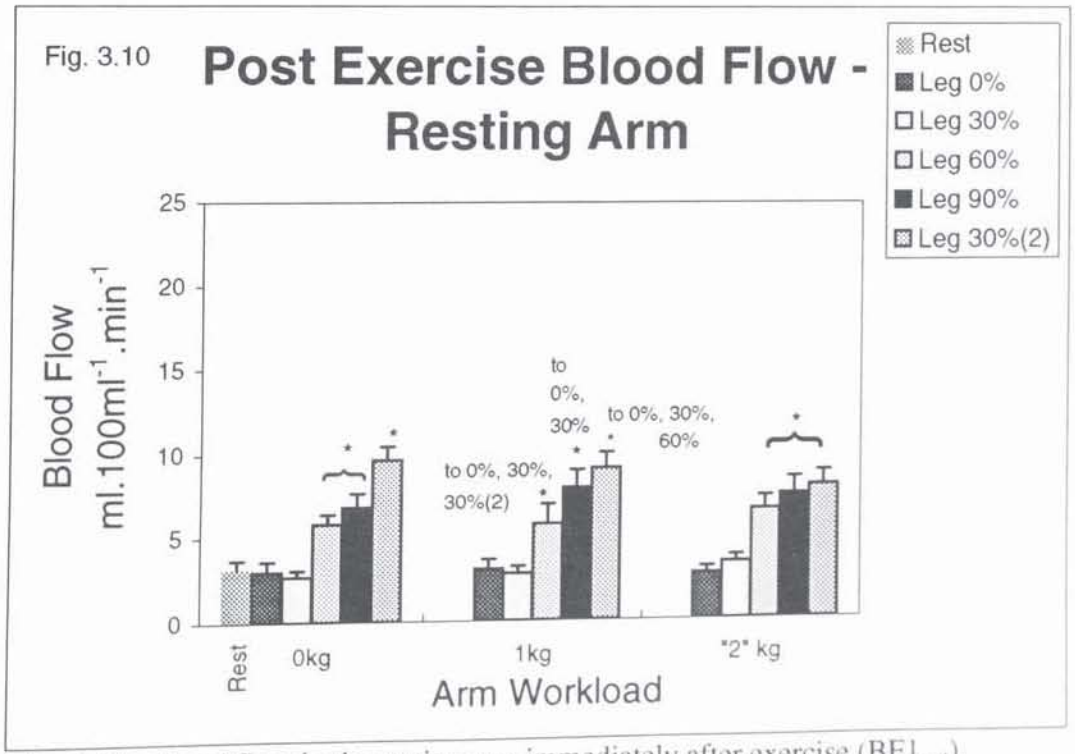


Fig. 3.10: Blood flow in the resting arm immediately after exercise ($BF1_{rest}$), plotted in relation to arm workload.

Fig. 3.11

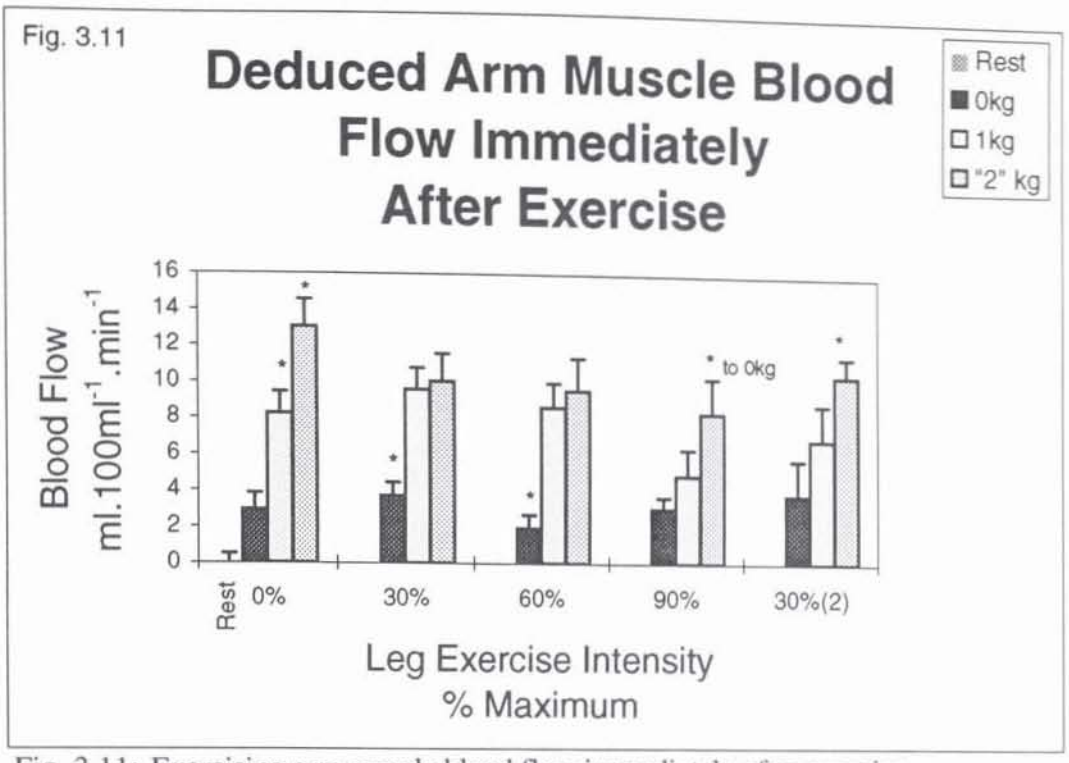


Fig. 3.11: Exercising arm muscle blood flow immediately after exercise ($BF1_{muscle}$ = exercising arm blood flow ($BF1_{ex}$) - resting arm blood flow ($BF1_{rest}$)), plotted in relation to leg exercise intensity.

Fig. 3.12

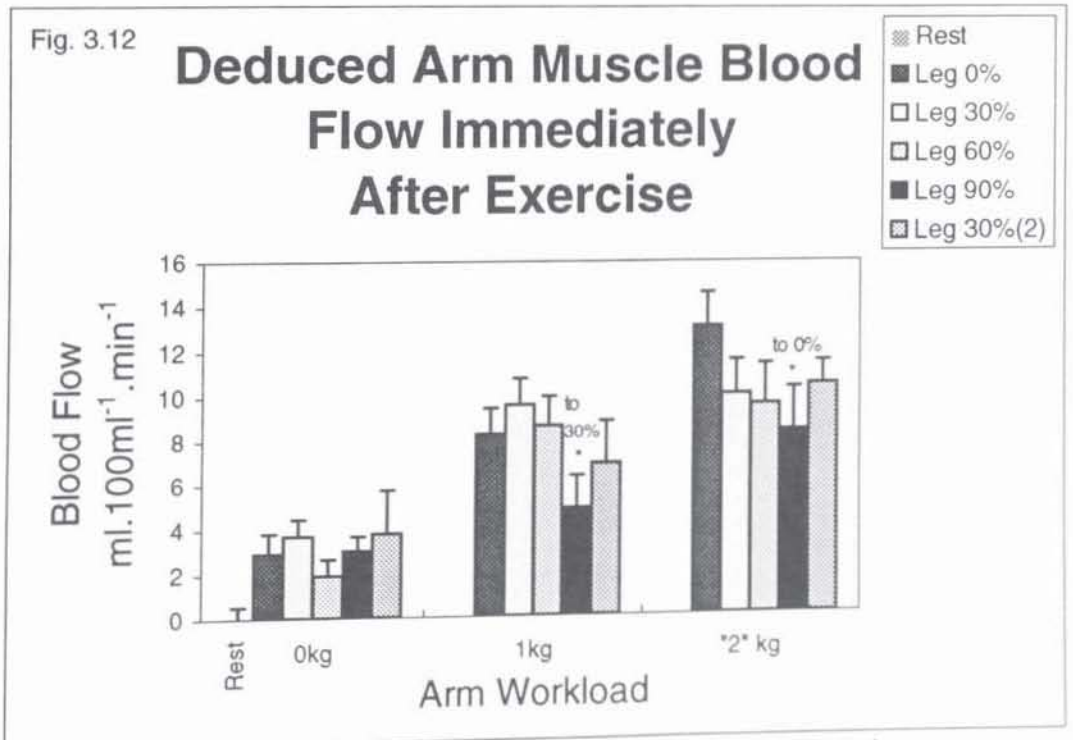


Fig. 3.12: Exercising arm muscle blood flow immediately after exercise ($BF1_{muscle}$ = exercising arm blood flow ($BF1_{ex}$) - resting arm blood flow ($BF1_{rest}$)) plotted in relation to arm workload.

Fig. 3.13

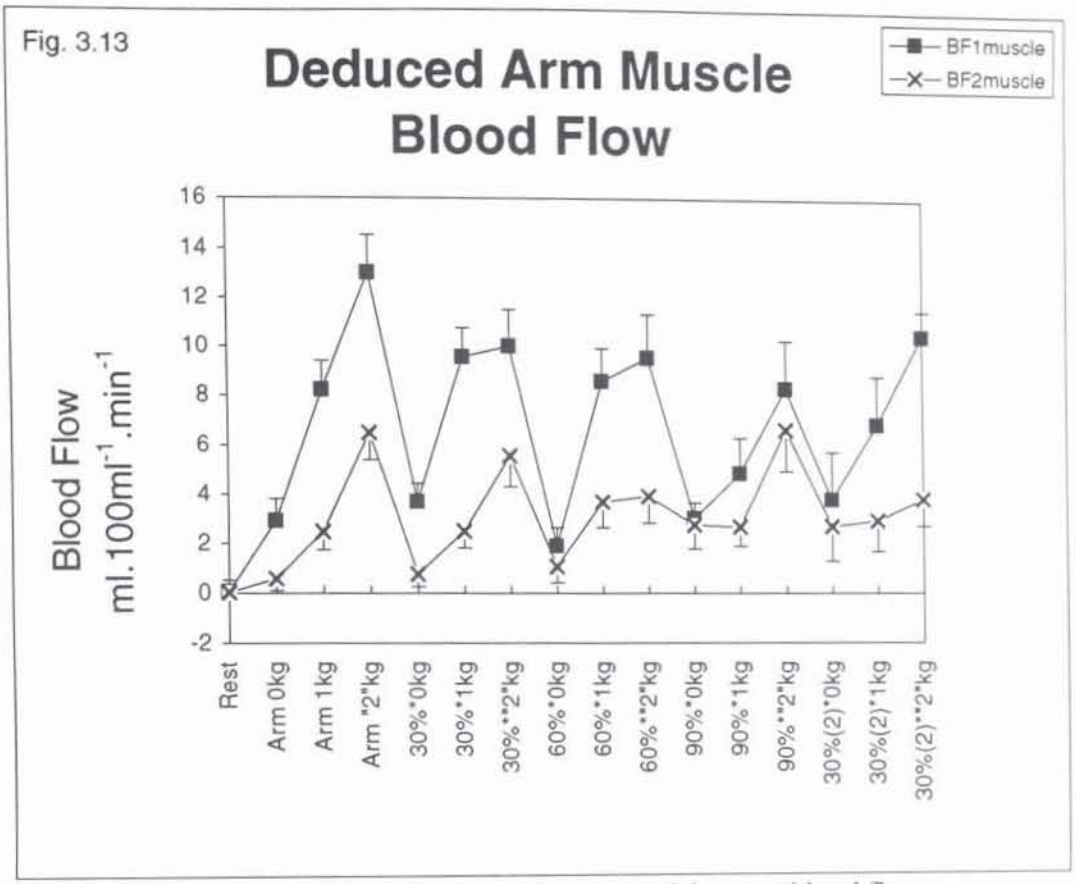


Fig. 3.13: Exercising arm muscle blood flow (exercising arm blood flow ($BF1_{ex}$, $BF2_{ex}$) - resting arm blood flow ($BF1_{rest}$, $BF2_{rest}$)), both immediately after exercise ($BF1_{muscle}$) and 2 minutes after exercise ($BF2_{muscle}$), plotted in relation to exercise bout.

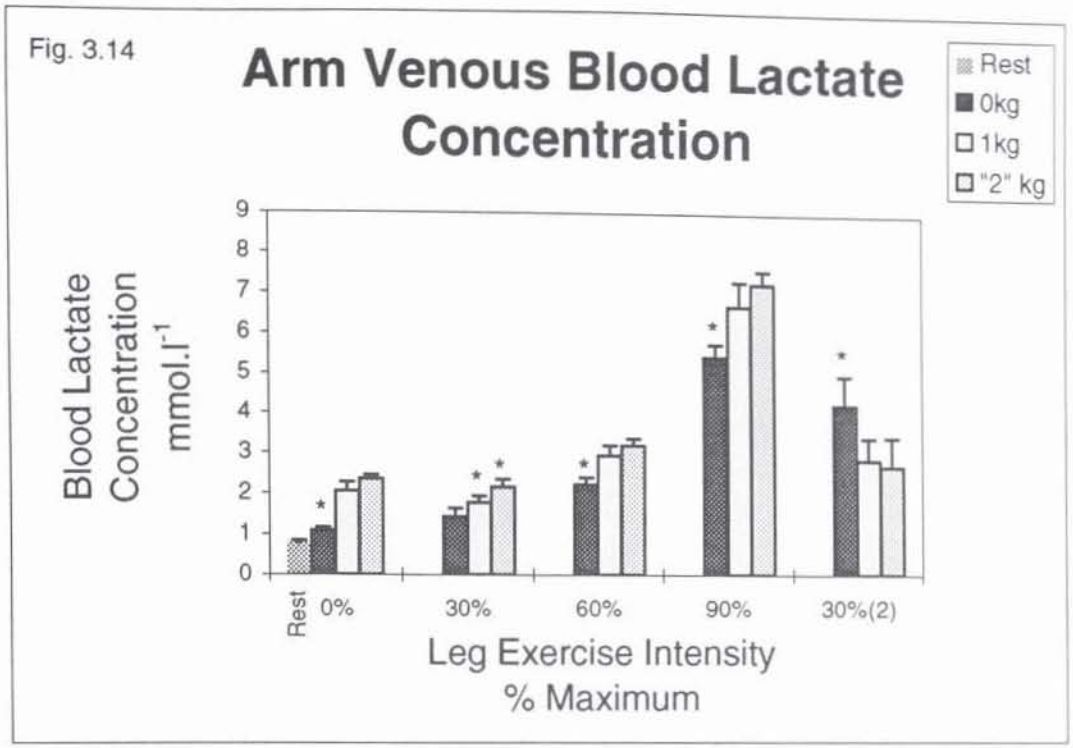


Fig. 3.14: Blood lactate concentration in blood sampled from the exercising forearm antecubital vein (Lac_{arm}), plotted in relation to leg exercise intensity.

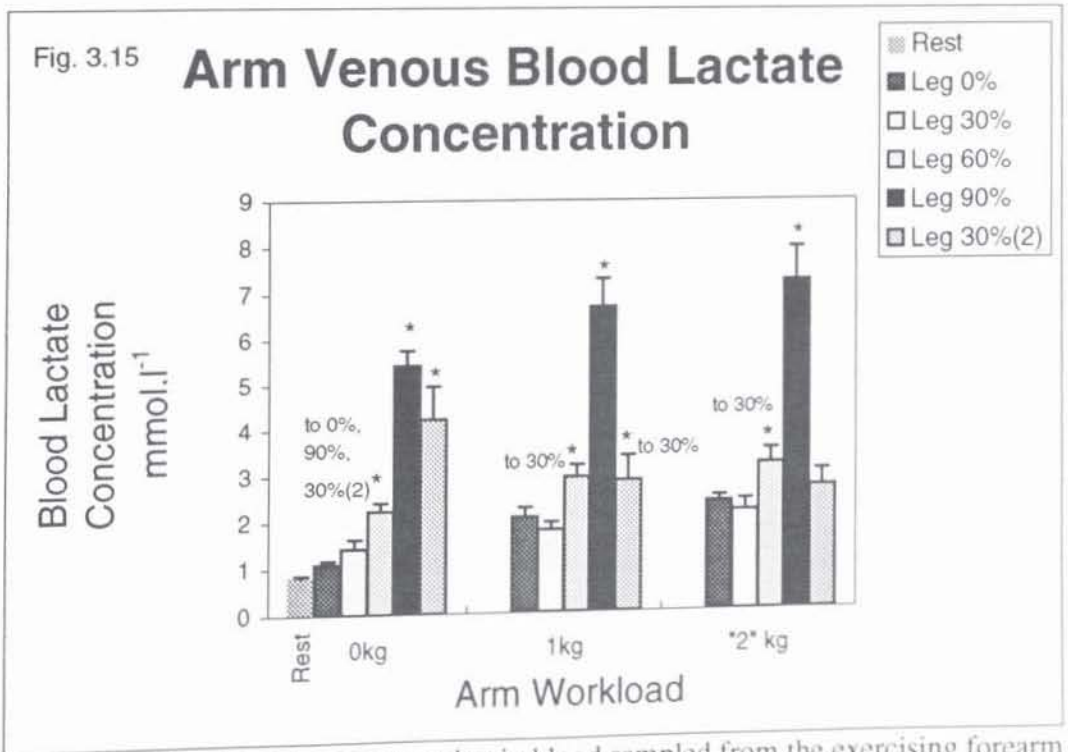


Fig. 3.15: Blood lactate concentration in blood sampled from the exercising forearm antecubital vein (Lac_{arm}), plotted in relation to arm workload.

Fig. 3.16

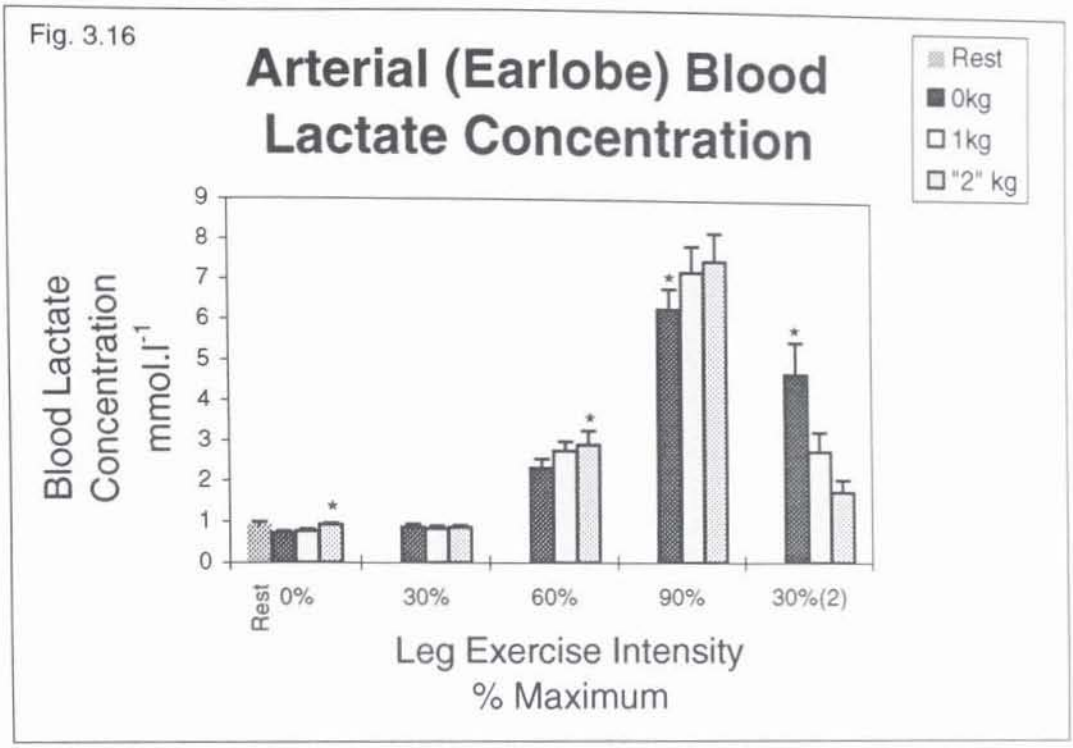


Fig. 3.16: Blood lactate concentration in blood sampled from the earlobe (Lac_{ear}), plotted in relation to leg exercise intensity.

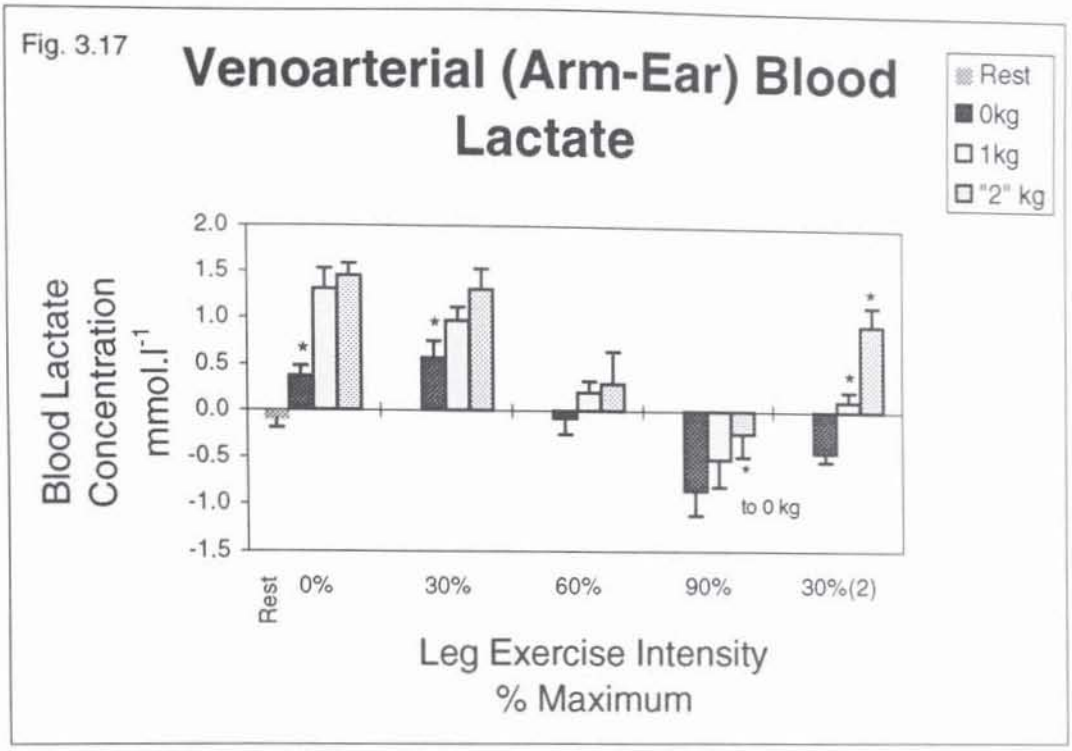


Fig. 3.17: Venoarterial blood lactate concentration difference (Lac_{arm} - Lac_{ear}), plotted in relation to leg exercise intensity.

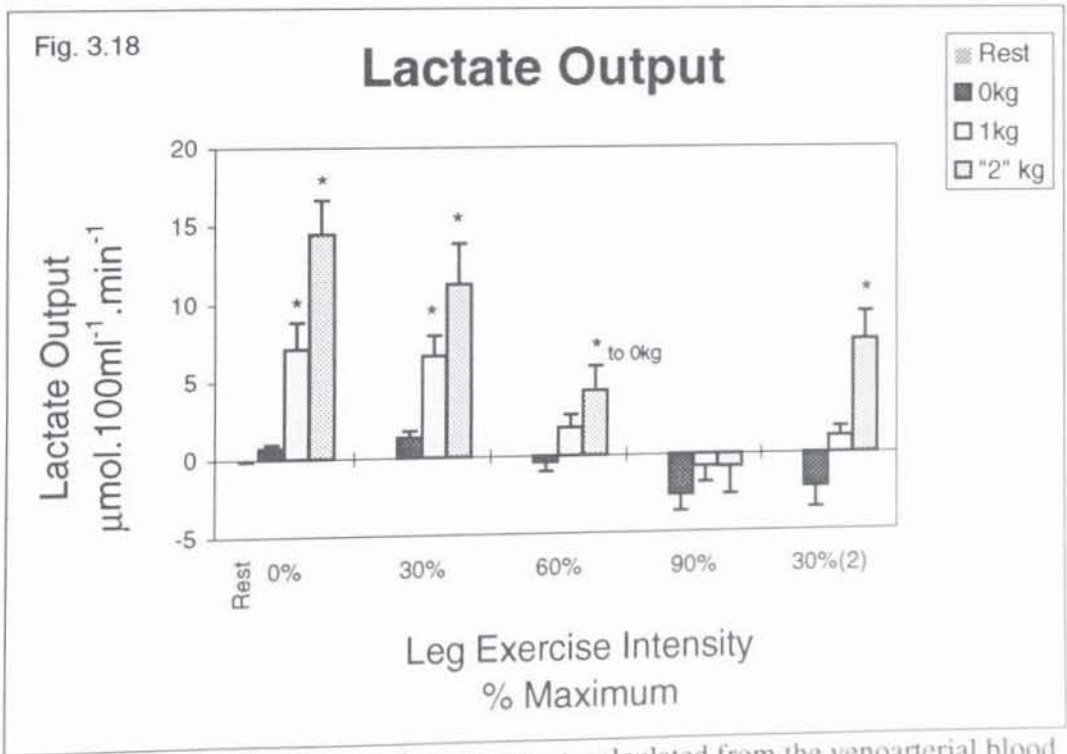


Fig. 3.18: Exercising forearm lactate output, calculated from the venoarterial blood lactate concentration difference, and the muscle blood flow at the time of blood sampling (average of $BF1_{muscle}$ and $BF2_{muscle}$), plotted in relation to leg exercise intensity.

Fig. 3.19

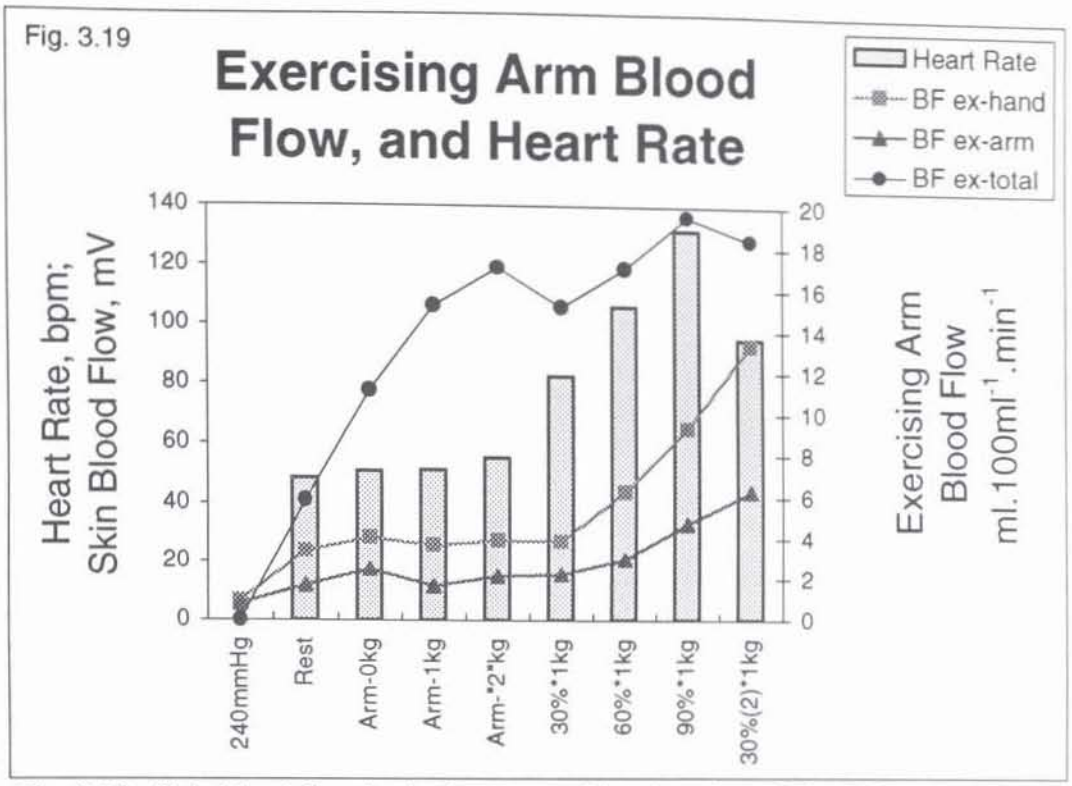


Fig. 3.19: Skin blood flow in the forearm and hand, and total blood flow in the exercising arm, and heart rate plotted in relation to exercise bout - Experiment 2.

Fig. 3.20

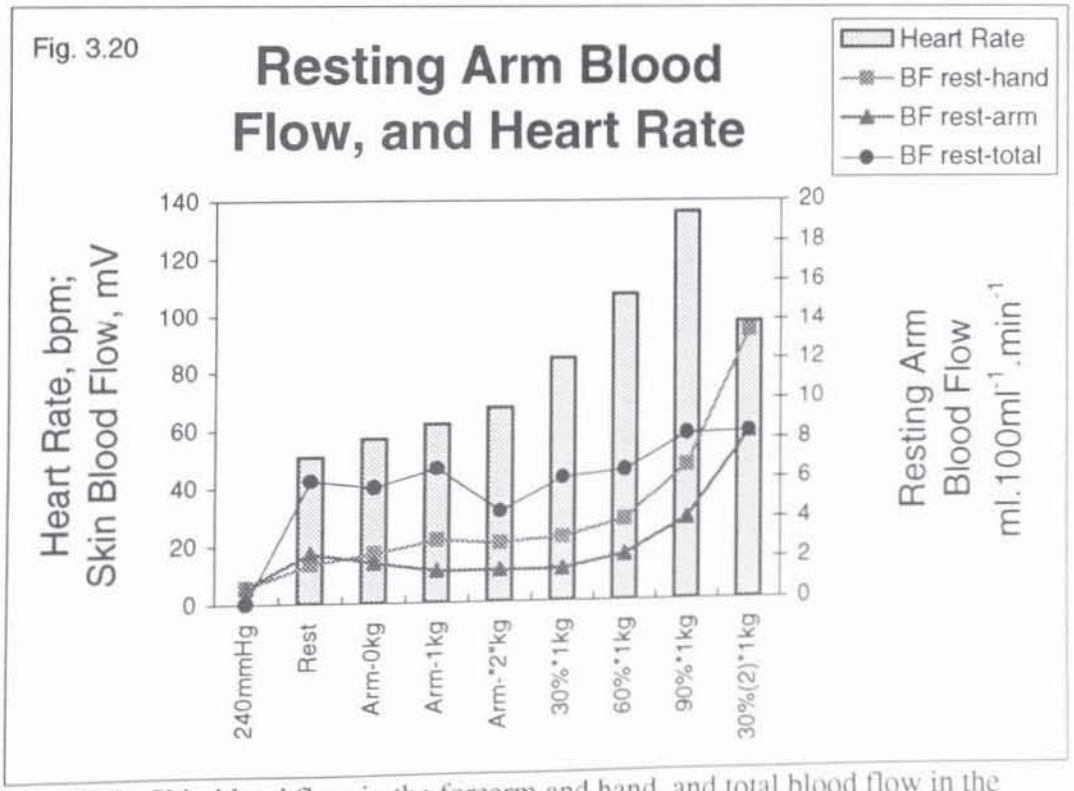


Fig. 3.20: Skin blood flow in the forearm and hand, and total blood flow in the resting arm, and heart rate plotted in relation to exercise bout - Experiment 2.

Fig. 3.21

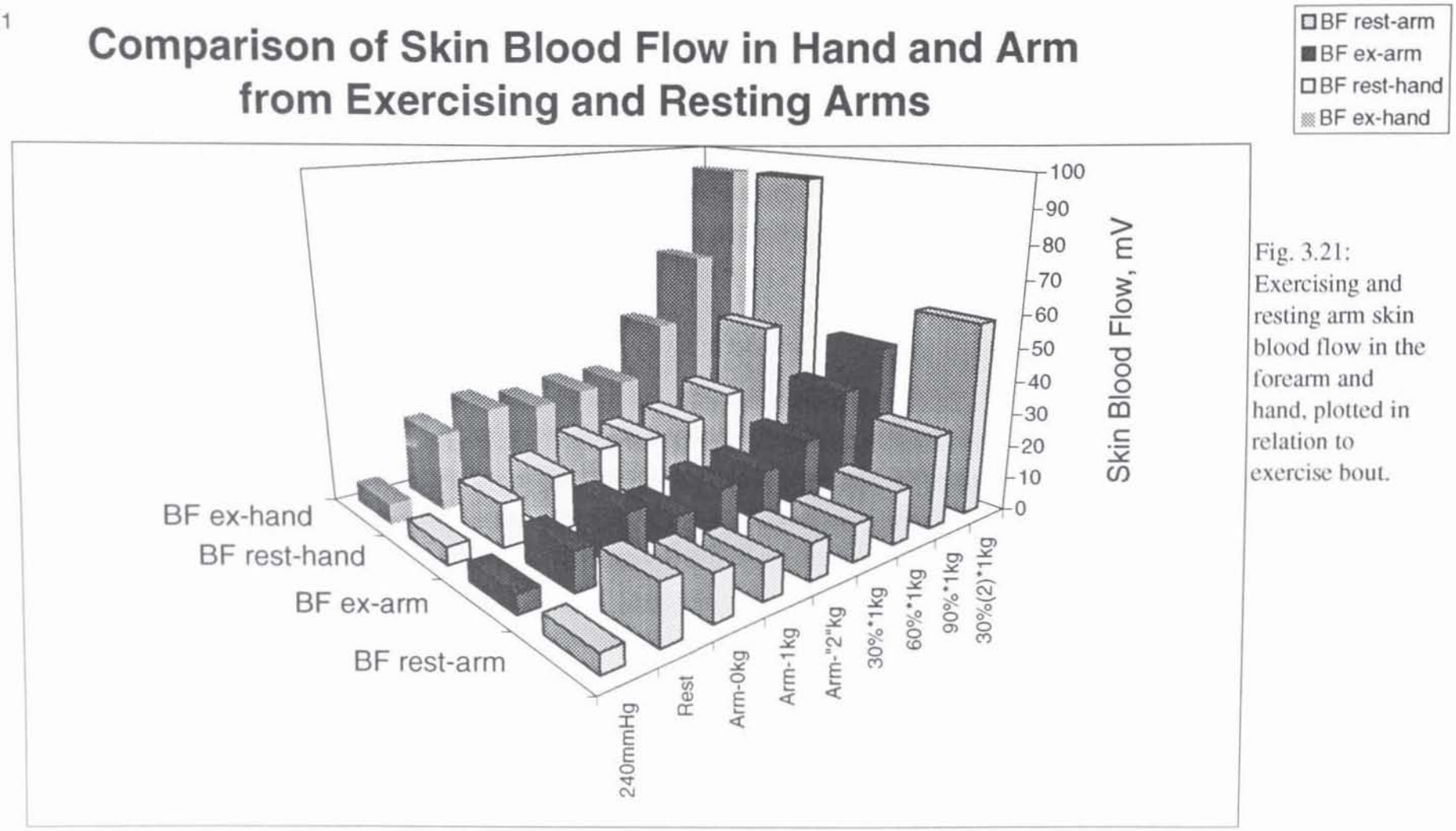


Fig. 3.21: Exercising and resting arm skin blood flow in the forearm and hand, plotted in relation to exercise bout.

DISCUSSION

The three increasing arm workloads were performed on their own, and superimposed on top of 4 different leg cycling intensities. The energy demand from the working forearm muscles could not change with leg activity, so the demand for blood and oxygen by the muscles must have remained constant. The resting arm blood flow indicated arm blood flow not attributable to exercise, including skin blood flow.

The original experiments were planned around upright cycling. Exercise in the supine position however offered many practical advantages. Venous occlusion plethysmography requires the studied limb to be at heart level to avoid venous pooling. When in the supine position, the subjects could simply relax their arms by their sides during venous occlusion, and rest them on the supine cycle ergometer surface. This meant that their arms were at heart level, and the relaxed position meant less chance of inadvertent arm movement during blood flow measurement. Resting their upper arm on the ergometer surface during forearm flexion exercise also meant the exercise was easier to control, and was localised as much as possible to the forearm flexor muscles. The long nature of this experiment also made subject comfort of paramount importance.

However, the supine position may have affected some of the results from that expected when the original experiment was planned around upright cycling. These differences are discussed in further detail later.

Blood Flow, Heart Rate

Immediately after exercise there was a significant increase in exercising arm blood flow with increasing arm workload (Fig. 3.5). There was less change with leg intensity (Fig. 3.6). Exercising arm blood flow after the easy arm exercise intensity (0 kg) bouts showed a gradual rise from the 60% leg exercise intensity onwards. There was no change in the resting arm blood flow with the exercising arm's workload, but a significant rise with increasing leg intensity (Figs. 3.9, 3.10). This again occurred later in the stages of the exercise, after the 60%, 90%, and 30%(2) bouts. This progressive rise in blood flow in the exercising and resting arms from the 60% leg stages onwards is possibly due to increased thermoregulatory flow to the skin.

Skin Blood Flow was assessed in Experiment 2 by using Laser Doppler Flowmetry. This generates a signal in mV, relating to the Doppler shift in the laser light reflected from the red blood cells - the greater the skin blood flow, the greater the quantity and speed of RBC's detected in the skin, and the greater the Doppler shift. Values can not be directly compared to those generated from Venous Occlusion Plethysmography (measured in $ml.100ml^{-1}.min^{-1}$), but estimates of the relative change in the skin/muscle blood flow can be made. Skin blood flow in both the exercising and resting arms, similar to the arm blood flow values in Experiment 1, increased progressively from the 60% leg intensity stages onwards (Figs. 3.19, 3.20, 3.21). These graphs also show that the skin blood flow changes

by similar amounts in both the exercising and resting arms after the various exercise combinations (Fig. 3.21).

Blood flow measurements taken after the blood sample, 2 minutes after exercise, generally all fell significantly from the initial measurements (Figs. 3.7, 3.8). This is consistent with the clearing of humoral and metabolic vasodilators, such as adrenaline, lactate, K^+ ions, H^+ ions, and hyperosmolality (Mellander & Johansson, 1968), which had been responsible for the exercise hyperaemic effect. However, following the most intensive bout of exercise ("2" kg arm workload at 90% leg intensity), the blood flow showed a slight, though non-significant increase from the first to the second reading in both arms. This is consistent with increased systemic production of vasodilators, due to it being the third bout of 90% leg effort, and perhaps also a state of partial ischaemia in the arm during exercise itself, due to blood 'stealing' by the legs in the 90% leg intensity bouts (Grant & Pearson, 1938; Mellander & Johansson, 1968).

The highest total blood flow values in both the resting and exercising arms occurred in the final 30%(2) bouts of exercise, particularly at the lower arm workloads (Figs. 3.5, 3.6, 3.9, 3.10). The easier leg exercise intensity will have caused a much reduced demand for blood from the legs compared to the previous stages. This, together with continued hyperaemia carried over from the previous heavier periods of exercise will all contribute to greater forearm perfusion and elevated blood flows. Also in the final 30%(2) bouts, the heart rate, which showed a slight, progressive increase with arm workload at each leg intensity but a much

greater increase with leg intensity, only fell from the 90% leg intensity level to mid-way between the initial 30% leg exercise bout and the 60% bout, and showed no significant difference over the three 30%(2) leg+arm bouts. The increased skin blood flow demand which showed a large increase at this leg exercise intensity (Fig. 3.21) is thought to be a principle factor in maintaining the high heart rates.

Heart rate increased during each 4 minute exercise bout. Values from the latter 2 minutes of leg+arm exercise were always higher than in the initial 2 minute leg-only period (Fig. 3.4). The added arm exercise may account for some of this increase. In an average 70 kg male, both legs cycling will use 14-16 kg of muscle (Saltin, 1986). In the same male, the average forearm muscle mass of a single forearm is 0.3 kg (Cooper *et al.*, 1955; Maughan *et al.*, 1984). Perhaps the majority, but certainly not all of this forearm muscle mass will be used in forearm flexion exercise.

At easy 30% leg intensity, adding the maximal "2" kg arm exercise caused an average increase of 7.7 bpm. During the 90% leg intensity bouts, adding "2" kg arm exercise caused an average increase of 10.0 bpm. Thus, adding 0.2-0.25 kg of extra muscle mass, working maximally, to 14-16 kg of already active muscle caused a 7-10 bpm increase in heart rate. This apparent disproportionately large heart rate increase on superimposing arm exercise has been observed before in exercise involving predominantly the upper body (Christensen, 1931; Åstrand *et al.*, 1968; Toner *et al.*, 1983). It has been ascribed to an increased isometric component of upper body exercise, less skeletal muscle pump activity possibly

affecting venous return, and a more dominating sympathetic tone during arm exercise. The slightly greater increase in the 90% x "2" kg results can be attributed to incomplete heat transfer, and a progressive increase in the fraction of cardiac output directed to the vasodilated skin as body temperature rose (Rowell, 1993a).

There is a much more pronounced increase in heart rate over the 3 bouts of both leg-only, and leg+arm exercise at the higher leg exercise intensities. This can similarly be attributed to incomplete heat transfer, and is consistent with cardiovascular drift (Rowell, 1993a). As leg exercise intensity increases, the skin blood flow will increase over the whole body to facilitate the elimination of the extra heat generated. The measured resting-arm blood-flows (Figs. 3.8, 3.9, 3.10) represent just a sample of this. Incomplete heat transfer will be achieved at each bout, so the subsequent bout at that intensity will require a further small increase in the cardiac output, and so will cause a slight further elevation in the heart rate.

The skin has a very high potential blood flow rate. A forearm blood flow of 12 $ml.100ml^{-1}.min^{-1}$ obtained due to heat stress, with no muscular activity, partitions the blood between the skin and the muscle in a ratio of 10:2 (Johnson, 1990). As the skin is only 9% of the forearm, this gives an equivalent blood flow to the skin of 100 $ml.100ml^{-1}.min^{-1}$. Conditions requiring significant skin blood flow will therefore cause more than trivial demands on the cardiovascular system.

The blood flow values obtained in this study agree with some of the maximal blood flow values quoted in the literature from venous occlusion plethysmography. The literature values can be quite variable however, suggesting inconsistencies with the technique. Williams & Lind (1979) obtained blood flow values of 16.9 $ml.100ml^{-1}.min^{-1}$ in the forearm after intermittent isometric handgrip contractions at 60% MVC (2 second contractions every 12 seconds, blood flow being measured in the 10 seconds of inactivity), with no occlusion of hand blood flow. Johnson & Rowell (1975) measured blood flow on an inactive arm at the end of 1 hour cycling at moderate intensity with occluded hand blood flow and got a maximum flow of 10 $ml.100ml^{-1}.min^{-1}$. These two values agree well with the maximum mean values in the present data of 18.5 $ml.100ml^{-1}.min^{-1}$ from the exercising arm (in the first blood flow reading after 30%(2) x "2" kg exercise), and 11.01 $ml.100ml^{-1}.min^{-1}$ from the resting arm (in the second blood flow reading after the 90% x "2" kg bout), both without occlusion of the hand blood flow. The slightly lower maximum value from Williams & Lind (1979) in the exercising arm compared to the present study may be expected due to their exercise being 2 seconds every 12, at 60% of MVC. The present study involved nearer maximum intensity exercise, continuous for 2 minutes. The inclusion of the hand blood flow could account for the differences in the inactive limb values.

However, Saumet *et al.* (1988) quoted values of 31.0 $ml.100ml^{-1}.min^{-1}$ in the forearm after very mild forearm exercise (simple opening and closing of the hand for 3 minute periods), though with a very large standard error of $\pm$ 9.95 $ml.100ml^{-1}.min^{-1}$. Earlier, Mellander & Johansson (1968) had quoted maximum

muscle blood flow values of 40-60 $ml.100ml^{-1}.min^{-1}$, although obtained from a variety of skeletal muscles. As the absolute blood flow values are not critical in this study, the results presented are assumed accurate and representative of changes in forearm blood flow.

Blood Stealing

Subtracting the resting arm blood flow values from the exercising arm values will give an indication of arm blood flow attributable to exercise (or Muscle Blood Flow - Figs. 3.11, 3.12). This shows that, in the case of the easy, 0 kg arm exercises, despite an apparent increasing blood flow with increasing leg intensity (Figs. 3.5, 3.6), the muscle blood flow remained largely unchanged. This suggests all the change in the exercising arm blood flow was due to increasing skin blood flow.

In contrast, after the 1 kg, and "2" kg maximal arm exercise bouts, relatively little variation was seen in the exercising arm blood flow with leg exercise intensity. The resting arm blood flow and the skin blood flow however, continued to increase with leg intensity. This led to a gradual reduction in muscle blood flow, reaching significant levels in the highest 90% leg exercise bouts.

This experiment was originally planned around upright cycling. Calculations showing that one third to one half of the total body muscle mass, when maximally

active, would tax the cardiac capacity were based on the physiological responses from upright individuals (Andersen & Saltin, 1985; Saltin, 1985; Saltin, 1986). As mentioned in the thesis introduction (p. 21), a reduction in muscle blood flow has been shown in upright exercise as the exercising muscle mass increases (Secher *et al.*, 1977; Andersen & Saltin, 1985; Saltin, 1985). Supine exercise has been shown to give higher stroke volume, lower heart rate, higher left ventricular end diastolic and end systolic volumes, and slightly lower $\dot{V}O_{2max}$ than upright exercise (Secher *et al.*, 1974; Johnson *et al.*, 1986; Poliner *et al.*, 1980). These will all contribute to give a superior forearm muscle blood perfusion in supine exercise, and offset any blood stealing expected from previous literature based around upright exercise responses. The actual measurement of the forearm blood flow may also have disguised the true response occurring during exercise. Measuring the blood flow as soon as possible after the cessation of exercise still allowed approximately 15 seconds to elapse before venous occlusion. The nature of the measurements precluded making them significantly earlier than this.

There is, however, evidence of blood stealing from the arms by the legs at the highest leg exercise intensity. Other research has indicated the persistent hyperaemia seen following the 90% leg exercise intensity bouts (Figs. 3.7, 3.8, 3.13) is consistent with the muscles having suffered oxygen lack (Mellander & Johansson, 1968). There is also a non-significant fall in muscle blood flow following the most intensive bouts of leg exercise (Figs. 3.11, 3.12, 3.13).

These all strengthen the conclusion that blood stealing may have occurred, and ischeamic exercising muscle conditions were seen, but only at the most intensive combinations of leg and arm exercise. Blood flow to the muscle in the resting arm may also be reduced during high intensity leg exercise, due to vasoconstriction in non-active regions diverting blood to metabolically active working muscles (Johnson & Rowell, 1975). When subtracting this value from the exercising arm blood flow, it would then lead to a slight elevation in the calculated exercising arm muscle blood flow value. This would further disguise any true 'blood flow stealing' in the exercising arm.

Blood Lactate

We may conclude that blood stealing, though less than expected when the experiment was first conceived, did occur, and may have been somewhat underestimated. It is therefore an extremely important finding that the forearm did not produce more lactate, but less (Figs. 3.17, 3.18). The traditional view of muscle metabolism, restated by Wasserman & Koike (1992) is that below the Aerobic Threshold (AT), energy metabolism is met entirely by aerobic metabolism, and lactic acid is not produced. Above AT however, aerobic ATP regeneration becomes inadequate to wholly sustain the level of muscular exercise and anaerobic metabolism must contribute, with consequent production of lactic acid. It is entirely contrary to this that, as shown in this chapter, working muscle can take up blood lactate at high whole body lactate concentrations (Fig. 3.17). If

the hypothesis of Hill, Sahlin, Wasserman and their respective colleagues were correct, namely that lactic acid is produced due to muscle tissue hypoxia, it would be an irreversible end-point metabolite. In the present study, the forearm muscles performed the same work with a low, and with a high concentration of lactate in its arterial blood supply. The switch from lactate release to lactate uptake reveals that lactate is in dynamic equilibrium with other pathways, and so indirectly supports the theory of Jöbsis *et al.* (1968), Connett *et al.* (1986), and Stainsby *et al.* (1989) that lactate production is unrelated to oxygen supply. It also agrees with the claims of Hubbard (1973), Brooks (1991), and Brooks *et al.* (1991) that lactate is an useful substrate, and an important means of distributing carbohydrate sources.

It has previously been shown that resting muscles can take up lactate, the uptake being correlated with arterial concentration (Stanley *et al.*, 1986; Gladden *et al.*, 1989; Chin *et al.*, 1991; Buckley *et al.*, 1993; Gutierrez *et al.*, 1993). Studies using radiolabelled lactate have shown exercising muscles to take up some lactate, but still to display net lactate release (Jorfeldt, 1970; Stanley *et al.*, 1986). In both these studies, lactate uptake was correlated with arterial lactate concentration. Both studies infused ^{14}C labelled lactate to a steady level. Jorfeldt measured lactate uptake during net lactate release across an exercising human forearm over 40 minutes of exercise. Stanley *et al.* (1986) did similarly over the legs in supine cycling, in four 6 minute incremental bouts, lasting 24 minutes.

Since 1986, several studies by Gladden and co-workers (e.g. Gladden, 1989; Gladden, 1991; Gladden *et al.*, 1994) in isolated preparations of the gastrocnemius-plantaris muscle group from a dog, have shown net lactate uptake across the exercising muscle with blood lactate infused to a concentration of 8-10 $mmol.l^{-1}$. They showed uptake rate increasing with contraction frequency, or $\dot{V}O_2$. These studies were all done on muscle continuously contracting over 30 minutes. The present study showed that easy, moderate, and hard exercising forearm muscles were all capable of net uptake of lactate over a much shorter period; however uptake was slowed, not accelerated, by higher work-rate in the uptake muscles.

Lactate Flux

The rate of lactate production/uptake can be quantified by combining the venoarterial blood lactate concentration differences and the average of the two muscle blood flow values (taken to represent the muscle blood flow at the time of blood sampling) from each exercise bout (Lac_{output} - Fig. 3.18). This gives an indication of the absolute amount of lactate taken up by the working muscles per unit time. The blood was sampled at 1 minute post exercise. Therefore only net lactate flux 1 minute post exercise can be absolutely concluded from the data, although results are inferred to similarly apply to the muscle during exercise. At 1 minute after the most intensive bouts of exercise, the arterial levels recorded were over 3 times greater than the maximum blood lactate concentrations in blood

sampled from the forearm vein after exercising alone (Figs. 3.14, 3.16). One can therefore assume that the gradient for lactate concentration between blood and muscle is always towards the forearm muscle in all the 90% leg intensity bouts.

The Lac_{output} values confirm that, as whole body lactate concentration increased, forearm lactate flux changed from net production to net uptake. It was however, only a significant net uptake after the initial, 90% x 0 kg bouts. The 90% x 1 kg, and 90% x "2" kg bouts showed an average net lactate uptake, but not significantly so. This is in contrast with the Lac_{v-a} results, which showed a significant uptake at both 90% x 0 kg, and 90% x 1 kg, and just not significant at 90% x "2" kg. With the elevated post exercise hyperaemia seen after the 90% bouts, and a negative Lac_{v-a} concentration difference, one may expect a large, significant net lactate uptake. However, when calculating the muscle blood flow at the time of blood sampling:

$$\left(\frac{(BF1_{ex} - BF1_{rest}) + (BF2_{ex} - BF2_{rest})}{2}\right)$$

isolated results from one subject in particular, showed the resting arm blood flow to be very close to the exercising arm blood flow, and at times greater than it. This gave an apparent negative muscle blood flow! When then combined with a negative Lac_{v-a} value, it would give an apparent net lactate output, when Lac_{v-a} was showing net uptake. This would obviously reduce the significance of the results somewhat. It then seems to suggest that the Lac_{v-a} results (Fig. 3.17) are

the best indicator of lactate flux in the exercising forearm in the present study. The general trend however, is clear. Net lactate output from the forearm predominates at low arterial blood lactate concentrations, which then falls as arterial lactate concentration rises, and becomes net lactate uptake above approximately 6 $mmol.l^{-1}$, despite the same arm exercise intensity.

This zero production, or significant net uptake continued at workloads that could not have been sustained for 30 minutes. It is doubtful, indeed, whether many subjects could have sustained their maximum arm workload for 3 minutes. The forearm muscle was thus working at an intensity which must have been producing lactate at near maximal rate when the arterial level was low; it nevertheless exhibited net lactate uptake when the arterial level was high.

Connett *et al.* (1986) calculated the contribution of glycolysis to energy generation, assuming lactate efflux was reflecting the steady-state glycolytic rate. They deduced that the contribution of glycolysis to the energy supply was only 1-2% in a maximally exercising (electrically stimulated) dog muscle. Energy generation may however involve lactate to a greater extent than estimated by Connett *et al.*, as their reasoning seemed to neglect the possibility of lactate being used as a carbohydrate source and an intermediate in energy generation. The net lactate uptake from the blood with increasing arterial blood lactate concentration in the present study may be occurring instead of glucose uptake, and being used for ATP regeneration. Results from at least one laboratory indicate that glucose uptake is suppressed in conditions of lactate uptake, as if the muscle uses blood

lactate preferentially (Richter *et al.*, 1988), though other workers have been unable to detect an interaction (Gladden *et al.*, 1994; Chin *et al.*, 1991). Glucose uptake by working muscles has been shown to be dependant on a concentration-induced increase in the availability of carriers for glucose (Ploug *et al.*, 1984; Richter *et al.*, 1985; Ploug *et al.*, 1987; Richter *et al.*, 1988). Lactate is a more oxidised, more broken down form of glucose, so would be quicker to be completely oxidised. It would enter metabolism at the lactate dehydrogenase level to be converted to pyruvate and then enter the Kreb's cycle as acetyl CoA. It would therefore require less enzymatic steps to be completely oxidised than blood glucose, which would enter at the start of glycolysis.

In the study by Richter *et al.*(1988), the reduction in glucose uptake by the leg muscles exercising at 80% $\dot{V}O_{2max}$, on adding arm cranking at 80% of their own $\dot{V}O_{2max}$, was accompanied by an arterial lactate increase from 2.1 $mmol.l^{-1}$ to 5.9 $mmol.l^{-1}$. The extra availability of lactate in the blood seems to cause it to be used in preference to glucose, as an energy substrate by the exercising muscle. If the lactate is assumed to be mostly oxidised (Jorfeldt, 1970; Hubbard, 1973; Chin *et al.*, 1991), it seems to more than compensate for the reduction in glucose uptake (Richter *et al.*, 1988). One can also speculate that muscle glycogen breakdown may also decrease in conditions of lactate uptake. The increase in Acetyl CoA, and therefore Citrate and ATP, may all inhibit phosphofructokinase. This will increase glucose-6-phosphate, which will in turn lead to inhibition of hexokinase and phosphorylase, and all contribute to slowing glycolysis (Ahlborg *et al.*, 1972).

Some studies have even shown a rise in arterial blood lactate that could not be accounted for by the lactic acid output of the contracting muscles (Stainsby *et al.*, 1985; Brooks, 1991; Brooks *et al.*, 1991). These studies suggest that there is an additional stimulant to lactate production, perhaps from adrenaline, causing it to be produced from tissues other than the active muscles, acting as an extra carbohydrate supply for energy generation.

Muscle size will have a major effect on lactate production. Spurway (1992) suggests that fully active, large muscle groups must have regions within them of O_2 deprivation. Lactate production could therefore still be from ischaemic regions of the legs. Muscles may be continually producing and consuming lactate, according to Brooks' lactate shuttle hypothesis (Brooks, 1991). In the present study, no measurements were made of the lactate gradient across the quadriceps muscles during the cycling. The net movement of lactate may simply be determined by the relative availability of lactate, glucose, and glycogen to the exercising muscles. Muscles seem to use their own endogenous stores of glycogen in preference to blood borne glucose, unless these stores are depleted (hypoglycaemia - Ploug *et al.*, 1984). High arterial concentrations of lactate will enhance lactate availability, and possibly cause it to be used in preference to glucose and glycogen (Essén *et al.*, 1973; Pearce & Connett, 1980). Energy substrates shift from glucose and triglyceride in muscles perfused with normal lactate perfusate, to glucose and lactate when perfused with high lactate perfusate (Chin *et al.*, 1991).

These results all agree with the present study. As arterial blood lactate concentration rose, lactate production from the muscle fell, and changed to net uptake. This was in conditions of potential decreased blood flow, when lactate production may be expected to rise if the oxygen-limitation theories, such as that from the Wasserman laboratory is correct (Wasserman & Koike, 1992).

Stanley *et al.* (1986) showed an increase in unidirectional lactate extraction, but still net lactate release, by quadriceps muscles working over 6 minute stages of increasing supine cycling exercise. No mention of the relative exercise intensity is made, but as two subjects failed to complete the final stage, and with a peak arterial blood lactate concentration of 9.08 $mmol.l^{-1}$, it may be assumed to have been close to maximal. Net lactate uptake has only previously been shown in exercising muscles in much longer duration exercise. Stainsby and co-workers (Stainsby, 1986; Stainsby *et al.*, 1991) showed net lactate production reverting to net lactate uptake in dog gastrocnemius-plantaris muscle in prolonged contractions of 30 minutes. Richter *et al.* (1988) showed a reversal from net lactate production in a single active quadriceps muscle, in the last minute of 15 minute exercise bouts, to net uptake when simultaneous arm cranking was added, raising the arterial lactate from 2.1 $mmol.l^{-1}$ to 5.9 $mmol.l^{-1}$. These studies suggested that in prolonged contractions, at elevated blood lactate levels, lactate uptake can occur.

The study by Gladden *et al.* (1994) showed that in dog gastrocnemius-plantaris muscle, the lactate uptake rate approaches a limit as arterial plasma lactate levels rise. Connett *et al.* (1986) had also suggested the existence of a saturable, carrier-mediated lactate transport system in muscle cell membranes. This all tied in nicely with the work of Roth & Brooks (1990a, 1990b) and Brown & Brooks (1994), who showed the existence of facilitated lactate transport across reconstituted rat skeletal muscle sarcolemmal membrane vesicles, which displayed saturation kinetics.

Lactate has been shown to be produced from both type I and type II fibres, but more readily from the latter (Connett *et al.*, 1986). Lactate uptake also occurs in both muscle types (Chin *et al.*, 1991), but has been shown to accumulate more in Type I muscle fibres than Type II fibres. This suggests either a preferential shuttling to Type I fibres, or a greater elimination rate from Type II fibres (Chin *et al.*, 1991). Type I muscles preferentially oxidise lactate to CO_2 and H_2O, but Type II muscles seem to direct lactate to glycogen synthesis (McLane & Holloszy, 1979; Pagliassotti & Donovan, 1990). Perhaps lactate may be produced from type II fibres, and oxidised by type I fibres with spare oxidative capacity, within the same muscle (suggested by Jorfeldt (1970), and the basis of Brook's Lactate Shuttle Hypothesis (1991)).

The muscle can still exhibit net lactate output whilst utilising some blood borne lactate, or may possibly switch to net lactate uptake if the arterial lactate concentration is high enough. Adrenaline has also been suggested to be involved

in the uptake of lactate by muscles, as well as possibly being responsible for its production (McDermott & Bonen, 1992). Lactate production may tend to predominate in the first 15 minutes of exercise, before the type II fibres fatigue. In the 30 minute experiments lactate uptake has been shown to occur, possibly by aerobic fibres using blood lactate as well as, or in preference to glucose. The present study shows that if the arterial lactate is high enough, exercising muscles can switch to net uptake of lactate much sooner.

The balance of evidence is, therefore, that in exercising whole animals, and humans, the direction of lactate transport is determined by the availability of lactate in the blood, and therefore the muscle-blood lactate gradient. Lactate uptake can accompany and more than compensate for any reduction in glucose uptake (Richter *et al.*, 1988). Only hypoglycaemic muscles have been shown to exhibit blood glucose uptake and utilisation, perhaps suggesting an inward directed blood-muscle concentration gradient (Ploug *et al.*, 1984). In the present study, the peak arterial lactate levels in the blood perfusing the forearm will have been higher than the intramuscular lactate levels of the forearm muscle, so net uptake occurred. This was in exercise of short duration, at much higher intensities than employed in the previous studies mentioned above. The 4 minute durations of the leg cycling, and the 2 minute durations of arm exercise will not be long enough to fatigue the type II fibres, as is possible in the 30 minute studies, but net lactate uptake is still seen.

The data of the present investigation also suggest a threshold level of extracellular, or blood lactate concentration for each arm workload, above which net lactate uptake by the exercising muscle occurs. During the easy, 0 kg arm workloads, an arterial lactate concentration over approximately 2.5 $mmol.l^{-1}$ will result in net lactate uptake by the muscle. For the most intensive bout, the arterial blood lactate concentration required for net uptake to occur rises to approximately 7 $mmol.l^{-1}$.

$\dot{V}O_{2max}$

A trained muscle exhibits a higher $\dot{V}O_{2max}$ due to increased capillarisation and mitochondrial density, allowing greater muscle blood flow, and oxygen extraction (Saltin, 1985). Stainsby *et al.*, (1995) showed that $\dot{V}O_2$ in a muscle increases to a point, $\dot{V}O_{2max}$, then decreases as stimulation/contraction frequency increases further, due to a reduction in muscle blood flow. They suggested regional blood flow variations may exist which are poorly revealed in analysis of whole muscle venous blood pO_2 measurements. They reduced blood flow to an exercising dog gastrocnemius-plantaris muscle by means of a pump to initiate ischaemia, and showed that $\dot{V}O_{2max}$ and the oxygen diffusion conductance (DCO_2) decreased, but the venous oxygen potential (PVO_2) remained constant (i.e. the blood pO_2 was unchanged, but reducing the blood flow reduced the supply of oxygen available, so reduced the $\dot{V}O_{2max}$).

Lactate is produced in increasing quantities as exercise intensity increases, and muscles exercise nearer their $\dot{V}O_{2max}$. In these present experiments, there was some evidence that the blood flow/oxygen supply to the exercising forearm muscle had been reduced in maximal exercise. Applying the findings of Stainsby *et al.* (1995) to this study, the DCO_2 may be reduced, but other than possibly some exercise induced hypoxaemia (Dempsey *et al.*, 1984; Powers & Williams, 1987) the arterial oxygen supply (PVO_2) would remain constant. Any possible depression of the forearm $\dot{V}O_{2max}$ would only occur during the most intensive, 90% leg exercise intensity. The large increase in lactate produced by the large leg muscles at this stage, offset any changes experienced by the forearm muscle, which took up lactate, even at the highest arm workload. This would be when the arm must have been working nearest, or possibly above its $\dot{V}O_{2max}$. Studies have shown marked lactate efflux from autoperfused pure red dog gracilis muscle (Connett, Gayeski & Honig, 1986), and other pure aerobic tissues such as the heart (Gertz *et al.*, 1981) above a threshold work rate of approximately 50% $\dot{V}O_{2max}$. This lactate efflux was in situations with normal resting levels of lactate in the blood perfusate. The data from the present study suggests that if the arterial lactate concentration had been increased, then at some point the muscles would have taken up lactate, even when working at maximal intensity.

THE OXIDATION STATE OF ISOMETRICALLY EXERCISING FOREARM MUSCLE

INTRODUCTION

The previous chapter showed that, in conditions that would be likely to promote lactate production if lactic acid was produced due to oxygen starvation, lactate was in fact taken up by exercising muscle. This seems incompatible with the traditional view that lactic acid is produced as an obligatory endpoint of the glycolytic pathway, operating in conditions when the muscle is insufficiently supplied with oxygen. Rather, it brings to mind the contention that muscle with intact blood supply never becomes oxygen limited (Connett *et al.*, 1984).

In aerobic energy generation, oxygen is only used by the terminal enzyme of the electron transport chain (ETC), cytochrome oxidase. This enzyme reduces molecular oxygen to water using the reducing equivalents generated from glycolysis and the citric acid cycle. It is the point within the cell that any shortfall in oxygen supply would be most apparent. If the oxygen supply became limiting, as Wasserman & Koike (1992), and Katz & Sahlin (1987) maintain occurs at high intensity exercise to initiate lactate production, this enzyme would become more reduced. Oxygen concentrations do fall in muscle during progressive exercise, but if oxygen never reaches limiting values in muscle, will the oxidation state of the cytochrome oxidase change at all during exercise?

As exercise intensity increases, the exercising muscle has a greater energy demand. This is usually accompanied by an increased blood flow to the exercising muscle. During isometric handgrip exercise at intensities up to 15% of Maximum

Voluntary Contraction (MVC), blood flow increases to a steady state, and tension can be maintained for several hours (Lind & McNichol, 1967; Åstrand & Rodahl, 1986a). Other cardiovascular variables such as heart rate and blood pressure also increase during the exercise, and fall back to resting values within a minute of the end of contraction. Between 15% and about 40% MVC, blood flow increases throughout exercise, failing to reach a steady state. It then increases further on cessation of exercise, as if the increase during exercise is insufficient to meet the metabolic requirements of the muscle and clear accumulating vasodilators, and the accumulated blood flow debt is being repaid afterwards. The other cardiovascular parameters also increase continuously during exercise, but again fall back to resting levels rapidly at the end of contraction. At handgrip intensities over 40% MVC, blood flow actually decreases during exercise, due to the increased intramuscular pressure from the contracting muscle, but it still increases at the end of contraction. Cardiovascular parameters follow the pattern previously established. Blood flow in isometrically exercising forearm muscle is completely occluded at intensities over 70% MVC (Lind & McNichol, 1967). As isometric exercise intensity increases, the circulation is progressively compromised relative to the metabolic requirements of the contracting muscle (Edwards & Wiles, 1981), and the demand on anaerobic energy generating systems must be assumed to increase.

Another means of changing the demand on the anaerobic energy generating systems is to alter the inspired oxygen concentration (FIO_2). By performing similar non-occluding exercises during hyperoxic (100% inspired oxygen),

normoxic (room air, 20.93% inspired oxygen), and hypoxic conditions (12.2 ± 0.1% oxygen, equivalent to an altitude of approximately 4000m), different demands will be placed on the energy generating systems. If anaerobic energy generation is a simple function of O_2 supply, greater emphasis will be placed on the anaerobic system as the FIO_2 decreases, (Lundin & Ström, 1947).

The reader will recall from Chapters 1 and 2 that the oxidation state of human skeletal muscle can be monitored by near infrared spectroscopy (NIRS). This is a non-invasive technique that can measure changes in the tissue concentrations of deoxyhaemoglobin (Hb), oxyhaemoglobin (HbO_2), and oxidised cytochrome oxidase (CtOx), by the absorbance of four separate wavelengths of near infrared laser light. The respective absorbance changes are converted into concentration changes by using the extinction coefficients of each compound at the four wavelengths, and combining all the absorbance changes into a specific algorithm (Cope, 1991).

Using NIRS, the oxidation state of human flexor carpi radialis muscle was monitored in the present study during isometric exercise at several intensities, and whilst subjects breathed normoxic, hyperoxic, and hypoxic gas mixtures. The isometric exercise was also carried out under conditions of complete blood flow occlusion to the exercising forearm. This of course had the effect of isolating the contracting muscle, accelerating the ischaemic affects of isometric exercise, and further challenging the aerobic energy generating system of the muscle. Lactate

outflow was also measured by sampling blood from the antecubital vein of the exercising forearm in some subjects.

If muscle metabolism is really determined by oxygen supply, and anaerobic energy generation is due to oxygen deprivation, the terminal electron acceptor of the ETC, cytochrome oxidase, will become more reduced as exercise intensity increases and inspired oxygen falls. This will promote anaerobic metabolism and lactate production. If, as now suspected, lactate production is unrelated to oxygen supply, then the question remains whether the oxidation state of the active muscle, and of its cytochrome oxidase in particular, will change at all during exercise.

Experiments were performed as follows:

Experiment 1 involved subjects performing isometric exercise at 40% of MVC, both with unobstructed blood access to the exercising muscle, and with complete occlusion by a brachial cuff around the upper arm. These two contractions were performed on three separate occasions, whilst breathing normoxic, hyperoxic (100% O_2), or hypoxic gas (12.2 ± 0.1% O_2). Muscle oxygenation, heart rate, and handgrip contraction force were monitored throughout each exercise.

Experiment 2 involved subjects performing isometric exercise at 20% MVC, 30% MVC, and 30% MVC again with an arterial occlusion cuff inflated around the upper arm, as in Experiment 1. Muscle oxygenation, and handgrip contraction force was monitored throughout exercise. In consenting subjects, peak post-

exercise blood lactate concentrations were also measured from the exercising forearm.

METHODS

All exercise testing took place in the Exercise Physiology Research Laboratory of Glasgow University (laboratory temperature: 20.9 ± 0.5 °C). The exercise involved in these studies was an isometric grip contraction on the handgrip dynamometer described in Chapter 2 (p. 52, Fig. 2.1), using the subject's dominant hand. Subjects initially performed 3 all-out voluntary grip contractions, with one minute of rest after each grip. The best score from these contractions was taken as 100% Maximum Voluntary Contraction (MVC). As noted above, various proportions of this maximum were used as the exercise intensities in the following studies. During the studies, the principle parameters measured were the grip strength throughout the period of contraction, recorded on computerised chart recording equipment, and the change in the concentrations of oxidised and reduced haemoglobin and in the oxidation state of cytochrome oxidase, using NIRS (NIRO-500, Hamamatsu Photonics KK, Japan; Chapter 2, p. 52). The optodes carrying the NIR signal were attached to the volar surface of the forearm over the belly of the flexor carpi radialis muscle, and covered by a light-proof black cloth to avoid excessive external light.

Subjects - Experiment 1

Eight male subjects volunteered for this study (age: 32.8 ± 4.0 yr.; maximum grip strength: 61.3 ± 5.9 kg). The study consisted of three identical exercise protocols,

repeated breathing normoxic, hyperoxic, or hypoxic gas mixtures. All 8 subjects carried out the normoxic and hyperoxic studies, but only 5 of the 8 (age: 34.8 ± 5.9 yr.; maximum grip strength: 59.5 ± 8.0 kg) were prepared to perform the hypoxic part.

Subjects - Experiment 2

Eighteen subjects (15 male, 3 female) volunteered for this study (age: 24.3 ± 1.4 yr.; maximum grip strength: 58.5 ± 4.9 kg). This study involved three different intensities of exercise, all performed breathing room air. In 8 of the subjects (6 male, 2 female; age: 24.9 ± 2.3 yr.; maximum grip strength 60.7 ± 6.4 kg) blood lactate concentration was also measured from a cannula inserted into the antecubital vein of the forearm.

SPECIFIC METHODS

Experiment 1

The subject was seated throughout, with the dominant arm used for the study, supported at chest height on a flat surface. The hand rested on the handgrip dynamometer at all times throughout the experiment, in such a position that in order to perform the exercise, the subject simply had to move his/her fingers, the

arm remaining still. This was done to reduce artefacts caused by excessive movement affecting the near infrared spectroscopy signal. All handgrip contractions were recorded as described in Chapter 2 (p. 52).

Throughout the experiments, the subjects breathed the respective gas mixtures from Douglas Bags, attached by a 1 metre length of non-kinkable, light weight respiratory tubing (Cranlea, UK) to a one way low resistance breathing valve (Hans Rudolf 2600, Kansas City, USA) supported by a headset (Hans Rudolf 2726, Kansas City, USA). The gas mixtures were room air (normoxic mixture), 100% oxygen (BOC, hyperoxic mixture), or room air with oxygen-free nitrogen (BOC) added to bring the oxygen concentration to 12.2 ± 0.1% oxygen (hypoxic mixture - final concentration determined using the gas analysis equipment of the Exercise Physiology Research Laboratory used to analyse Douglas Bag contents, Chapter 2, p. 42). The gas mixture stages were presented in randomised sequence so the subjects were unaware what mixture they were breathing. Heart rate was recorded every 5 seconds throughout by Polar Sports Tester, and the concentrations of oxidised and reduced haemoglobin, and the oxidation state of cytochrome oxidase were monitored throughout from the flexor carpi radialis forearm muscle using NIRS (Chapter 2, p. 52).

The intensity of exercise used in this study was 40% of MVC. The mouthpiece for breathing the various gas mixtures, and a noseclip, were fitted prior to a 5 minute period of rest, with the subject breathing room air and the NIRS signal recorded at 1 sample per second. The subject was then switched at the end of an expiration,

to breathing from a Douglas Bag for a further 1.5 minutes' preparation. This was followed by a 2 minute period of contraction at 40% of MVC, while the subject continued to breathe from the same bag. Feedback during contraction was given by a digital readout of the force produced, displayed in front of the subject, and effort was reinforced by verbal encouragement. Despite this, two of the subjects could only manage 1.5 minutes' contraction. In these subjects, all the other durations were as described. At the end of the contraction period, the subject relaxed his/her grip, but was constantly reminded to maintain the hand position on the dynamometer, and recovery was recorded for 1 minute, with the subject still breathing from the Douglas Bag. Then the subject was switched back to breathing room air, and the mouthpiece and noseclip removed, still maintaining the hand and arm position on the dynamometer.

With the subject breathing freely, a 5-10 minute period of recovery was allowed until the NIRS signals reached a stable level. During this period, arm movement was constantly discouraged. The second phase of the test, still breathing the same gas mixture, used the same protocol as the first part, with the addition of a further minute of rest. This was introduced after the 1.5 minutes of rest breathing from the Douglas Bag before grip contraction. During this minute, blood flow to the exercising muscle was completely occluded by inflating a cuff around the upper arm to 240 mmHg. Subjects were still breathing from the Douglas Bag during this period. Occlusion was maintained for this 1 minute prior to contraction, throughout the 2 minute exercise period, and for 30 seconds immediately after contraction. The pressure was then released and the NIRS signal recorded for a

further 30 seconds, with the subject still breathing the gas mixture from the Douglas Bag. The subject was then switched back to breathing room air and the experiment terminated.

Subjects performed the above on 1-2 further occasions, depending on whether they volunteered to breathe all three gas mixtures, or only normoxic and hyperoxic mixtures. Exactly the same procedure was carried out irrespective of the gas mixture breathed.

Experiment 2

The handgrip dynamometer, mode of use, and measurement of MVC were all as previously described. In this case, 20% and 30% of MVC were calculated as the isometric exercise intensities to be used in the main experiment. The concentrations of oxidised and reduced haemoglobin, and the redox state of cytochrome oxidase were again monitored by NIRS, but using larger, 20 mm optodes still attached to optical fibres, which gave a slightly steadier signal than the 8 mm ones used previously. They were again affixed over the belly of the volar surface of the flexor carpi radialis forearm muscle.

In 8 subjects, lactic acid production from the exercising muscle was monitored from blood samples taken from a cannula in the antecubital vein of the forearm. The cannula was inserted and a 5 minute rest period was allowed before blood

was sampled to determine a resting blood lactate value (as previously described, Chapter 2, p. 44). Blood samples were analysed using an Analox GM7 lactate analyser (Analox Instruments Ltd., UK).

A 5 minute rest period, with the arm relaxed and stationary in the experimental position, was allowed in all subjects prior to exercise. The exercise consisted of three 2 minute periods of isometric contraction, performed at different intensities. In a fixed order, they were 2 minutes at 20% MVC, 2 minutes at 30% MVC, then 2 minutes at 30% MVC again, but with arterial occlusion by the brachial cuff. This was similar to Experiment 1, but due to complaints of discomfort from subjects in the previous study, the cuff was inflated at the start of the grip period, to 200 mmHg instead of 240 mmHg, and released immediately on completion of 2 minutes handgrip contraction.

Where venous blood lactate concentrations were being measured after each exercise period, the first blood sample was taken 30 seconds after the end of exercise, and samples followed every minute thereafter until the blood lactate concentration had peaked. A further resting sample was taken just before the start of each successive period of exercise, after approximately 5-10 minutes of rest. As in the previous experiment, rest was continued until the NIRS signals had stabilised, before the next period of contraction followed. Two subjects failed to make 2 minutes in the third phase of contraction, at 30% MVC with arterial occlusion, managing 1.5, and 1.75 minutes respectively. Neither of these subjects

were part of the study measuring blood lactate concentration from the forearm antecubital vein. All other durations were kept constant.

RESULTS

Analysis

The near infrared data were analysed in the same way in both experiments. Data were recorded at a rate of 1 sample per second (Experiment 1), or 2 samples per second (Experiment 2). Data were analysed by looking at the rates of change in the NIR signals over the period of exercise (Fig. 4.1). From the NIRS trace recorded during exercise, the average of a 4-second period of data was taken from the beginning of exercise, and from a 4-second period at the end of exercise, and the difference between the two values calculated (Figs. 4.1, 4.2). This value was then divided by the time between the two sampling points, to form a 'rate of change' value. Care was taken to avoid the periods at the very beginning and end of exercise, when the application or release of contraction caused sharp movement artefacts in the NIRS signal.

The 2 minute analysis period was subsequently split further into the first 30 seconds, and the subsequent 1.5 minutes. The same 4 second averaged value was used as the end of the 30 second analysis period and the start of the 1.5 minute analysis period (Fig. 4.1). Again, the difference between the two 4-second values was divided by the time between the averaged points, to obtain rates of change.

Isometric handgrip force was also analysed in a similar manner, by calculating the average of 4 second periods of data from the beginning and end of the handgrip

trace during the period of contraction, and calculating the difference between (Fig. 4.3).

Heart rate was analysed by averaging the last 30 seconds of data from the respective stages of the exercise bout.

Statistical Analysis

A 2-way analysis of variance test was used to analyse the data. Confidence intervals were calculated using Bonferroni follow-up tests. Statistical significance was accepted at the 5% level. In Experiment 1, statistical analysis was performed on the 5 subjects who performed all 3 tests (normoxic, hyperoxic, and hypoxic conditions). The 3 subjects who only carried out the normoxic and hyperoxic studies showed the same kinds of results on those two tests as the other subjects. Their data was combined with the other 5 subjects, for purposes of illustrating the data graphically (Figs. 4.4 - 4.11). This decreased the Standard Error values, from an average of 2.9 when n = 5, to 2.6 when n = 8. In Experiment 2, statistical analysis was carried out on the data from all the subjects. Statistical significance was taken at the 5% level, and illustrated graphically by an asterisk (*) relating to each group of values.

Experiment 1

Expt. 1: Isometric Handgrip Force

Subjects were asked to contract at 40% MVC, equivalent to a mean force of 23.0 ± 1.4 kg. A decrease in handgrip force over the contraction period was seen in some subjects (Figs. 4.3, 4.4). The decline in force in the cuff-occluded studies was significantly greater than in the non-occluded studies. The effect of inspired oxygen concentration was not statistically significant; the hyperoxic conditions however, generally produced a lower drop in grip contraction force than the normoxic and hypoxic conditions, in both the non-occluded state (mean force decline in hyperoxia = 1.3 ± 0.7 kg, compared to normoxia = 3.7 ± 1.7 kg, and hypoxia = 1.5 ± 0.9 kg), and during occluded contraction (force decline in hyperoxia = 6.8 ± 2.0 kg, compared to normoxia = 9.1 ± 2.8 kg, and hypoxia = 9.4 ± 2.6 kg).

Expt. 1: Heart Rate

Heart rates before, during, and after the grip period were always higher - in most instances significantly so - during hypoxia than during the other conditions (Fig. 4.5, Table 4.1).

Table 4.1: Heart rates recorded during the different stages of isometric study breathing different FIO_2. (DB = breathing from Douglas Bag; No Cuff = not involving cuff occlusion; ± Cuff = before, during, or after cuff occlusion; all values are Mean ± SE; * = significantly different to hyperoxia, † = significantly different to normoxia).

Period	Time (min)	Hypoxia		Normoxia		Hyperoxia	
		No Cuff	± Cuff	No Cuff	± Cuff	No Cuff	± Cuff
Rest - Pre DB	**5.0**	**67.2** ± 5.6	**67.6** ± 6.4	**69.4** ± 4.9	**66.1** ± 4.3	**67.2** ± 3.2	**67.0** ± 4.0
Rest - DB	**1.5**	**71.1** ± 5.9	**70.1 *** ± 6.8	**67.6** ± 4.0	**65.6** ± 3.6	**66.0** ± 3.6	**65.3** ± 3.9
Rest- Occlusion	**1.0**	-	**75.6 *†** ± 6.8	-	**66.4** ± 4.1	-	**65.6** ± 4.3
Grip	**2.0**	**84.9 *** ± 5.4	**87.9** ± 7.1	**81.0** ± 2.8	**81.0** ± 3.5	**79.2** ± 3.3	**82.9** ± 5.4
Post Grip	**1.0**	**76.3 *** ± 6.7	**72.8 *†** ± 6.8	**73.1** ± 3.2	**66.0** ± 4.6	**65.7** ± 2.9	**63.7** ± 4.1
Release of Occlusion	**0.5**	-	**67.8 †** ± 8.2	-	**61.3** ± 4.4	-	**63.1** ± 4.7
Mean	-	**74.8**	**73.6**	**72.8**	**67.7**	**69.5**	**67.9**

Expt. 1: Deoxyhaemoglobin (Hb), Oxyhaemoglobin (HbO_2)

The gas mixtures had small, mostly non-significant effects on the deoxyhaemoglobin (Hb) and oxyhaemoglobin (HbO_2) concentrations in the periods of rest prior to exercise, and prior to occlusion (Figs. 4.6, 4.7).

In the 1 minute of occluded rest, the Hb and HbO_2 concentrations rose and fell respectively (Figs. 4.6, 4.7). The hyperoxic conditions produced a significantly lower rate of Hb increase (13.8 ± 1.3 $\mu mol.min^{-1}$), compared to the normoxic and hypoxic conditions (17.6 ± 2.0 $\mu mol.min^{-1}$ and 18.4 ± 1.2 $\mu mol.min^{-1}$ respectively), but no significant effect was seen in the HbO_2 concentrations. The overall average rate of Hb increase during the occluded rest, (average = 16.6 ± 1.5 $\mu mol.min^{-1}$) was greater than the rate of HbO_2 decline (average = 11.1 ± 1.9 $\mu mol.min^{-1}$). This pattern of greater rate of Hb change than HbO_2 was generally repeated throughout the study.

During exercise, the Hb concentrations again became significantly elevated - markedly so in occluded conditions. The non-occluded study showed no significant effect due to the breathing mixture. Under cuff-occlusion, the rate of Hb change during the hypoxic condition (8.2 ± 6.6 $\mu mol.min^{-1}$) was significantly lower than occurring in both the other conditions (21.0 ± 2.7 $\mu mol.min^{-1}$ and 20.0 ± 2.0 $\mu mol.min^{-1}$ in normoxia and hyperoxia respectively).

The HbO_2 concentrations also changed during exercise, becoming significantly reduced. This effect was however, largely in the occluded exercise periods (Fig. 4.7). In the non-occluded exercise, only the hypoxic condition produced a significant fall in HbO_2 concentration (5.1 ± 3.3 $\mu mol.min^{-1}$). Under cuff-occlusion, all conditions showed a fall in HbO_2, but the hypoxic conditions showed a smaller rate of change (8.1 ± 3.2 $\mu mol.min^{-1}$) compared to the normoxic condition (12.3 ± 1.8 $\mu mol.min^{-1}$) and the hyperoxic condition (13.3 ± 2.7 $\mu mol.min^{-1}$).

Generally more of the change in Hb and HbO_2 occurred in the first 30 seconds of the 2 minute contraction period than in to the last 1.5 minutes (Figs. 4.8, 4.9). The gas mixtures had small effects in this analysis, most of them non-significant. However, the rate of Hb concentration change during the hyperoxic condition became significantly greater than during the hypoxic condition, in the last 1.5 minutes of exercise under occlusion (Fig. 4.8).

In the recovery period after non-occluded exercise, the Hb and HbO_2 concentrations fell and rose respectively (Figs. 4.6, 4.7; average rate of Hb fall = 8.3 ± 3.3 $\mu mol.min^{-1}$, HbO_2 rise = 4.7 ± 3.4 $\mu mol.min^{-1}$). By contrast, in the cuff-occluded recovery period, the Hb and HbO_2 continued to rise and fall respectively. On release of arterial occlusion, the Hb and HbO_2 concentrations showed sharp rapid decreases and increases respectively, as the blood flowed back into the arm (average 119.8 ± 13.5 $\mu mol.min^{-1}$ Hb fall, and 107.8 ± 11.1

$\mu mol.min^{-1}$ HbO_2 rise). Inspired oxygen concentration had no significant effect in any of the periods of recovery after grip contraction.

Expt. 1: Cytochrome Oxidase (CtOx)

In the periods of rest breathing the different gas mixtures, in the non-occluded state or under arterial occlusion no significant change was seen in the amount of oxidised cytochrome oxidase (CtOx, Fig. 4.10). The CtOx only changed at a significant rate during the 2 minute contraction period under hypoxic occlusion. In this instance, contrary to intuition and any notion of oxygen being the rate-limiting factor in muscle metabolism, over the 2 minutes the CtOx became significantly more oxidised (2.2 ± 0.9 $\mu mol.min^{-1}$).

Breaking down the 2 minute contractions into two phases (Fig. 4.11), we see that CtOx generally became more reduced during the first 30 seconds, the trend coming close to significance during the unoccluded bouts of both hypoxic and normoxic conditions relative to hyperoxia. In the subsequent 1.5 minutes, there was largely no change. However, in the subsequent 1.5 minutes of the hypoxic, flow-occluded muscle the CtOx changed at a significant rate, its CtOx becoming significantly oxidised.

In the period after contraction (Fig. 4.10), the CtOx again became more oxidised under cuff-occlusion, compared to normal blood flow. This is marked in the

hypoxic condition, in which the CtOx redox state became further oxidised at a significantly greater rate (7.31 ± 2.7 $\mu mol.min^{-1}$) compared to the normoxic and hyperoxic conditions (2.8 ± 1.5 $\mu mol.min^{-1}$ and 0.5 ± 1.4 $\mu mol.min^{-1}$ respectively).

On release of arterial occlusion, the NIRS signals were generally more noisy, but a general trend was clear. CtOx reverts to becoming reduced, with the hypoxic limb (11.3 ± 6.7 $\mu mol.min^{-1}$) showing this response most rapidly, but only at a significantly greater rate compared to the hyperoxic limb (2.3 ± 1.7 $\mu mol.min^{-1}$).

Experiment 2

Expt. 2: Isometric Handgrip Force

The isometric handgrip force decline over the 2 minute contraction period became greater with increasing exercise intensity (Fig. 4.12). Subjects were asked to exercise at 20% MVC (mean force = 12.7 ± 0.8 kg) and 30% MVC (mean force = 19.0 ± 1.2 kg). At the easiest grip intensity there was a slight tendency to grip harder at the end of 2 minutes (0.1 ± 0.2 kg). This changed to a small, 0.9 ± 0.4 kg decline at 30% MVC intensity, and a 2.5 ± 1.0 kg decline in the cuff-occluded 30% bout of exercise, significantly greater than the 20% result.

Expt. 2: Deoxyhaemoglobin (Hb), Oxyhaemoglobin (HbO_2)

Very little change in the Hb and HbO_2 concentrations were seen in the forearm during the 20% and 30% MVC intensities of exercise with non-occluded blood flow to the muscle (Figs. 4.13, 4.16). The 30% bout in the occluded state however, showed a significant rise in the rate of Hb accumulation (13.4 ± 1.0 $\mu mol.min^{-1}$) and fall in HbO_2 (4.2 ± 1.1 $\mu mol.min^{-1}$, one subject illustrated in Fig. 4.2). There was no significant difference between the rates of change of Hb and HbO_2 within the 2 minute exercise period in the non-occluded bouts. There was however, a non-significant tendency for both the Hb and HbO_2 concentrations to become lower in the first 30 seconds, and then increase in the last 1.5 minutes of exercise (Figs. 4.14, 4.17). In the occluded bout of exercise however, the Hb increased and the HbO_2 decreased significantly over both the first 30 seconds and the last 1.5 minutes of the exercise.

These changes were reversed by cuff removal, from an increase in the rate of Hb accumulation (13.4 ± 1.0 $\mu mol.min^{-1}$) and decrease in HbO_2 (4.2 ± 1.1 $\mu mol.min^{-1}$) during the 2 minutes of occluded exercise (Figs. 4.13, 4.16), to a rapid decrease in Hb (23.4 ± 2.2 $\mu mol.min^{-1}$) and increase in HbO_2 (11.9 ± 2.6 $\mu mol.min^{-1}$) in the following minute (Figs. 4.15, 4.18). Very little effect was seen due to release of contraction in the non-occluded bouts of exercise.

The Hb and HbO_2 NIRS signals fluctuated during the rest periods between exercise bouts. When using the larger optodes however, the fluctuations of Hb

and HbO_2 were seen to be in equal and opposite directions (Fig. 4.24). The extent of this fluctuation varied between subjects, and caused some variation in the duration of the rest periods whilst waiting for apparent stability.

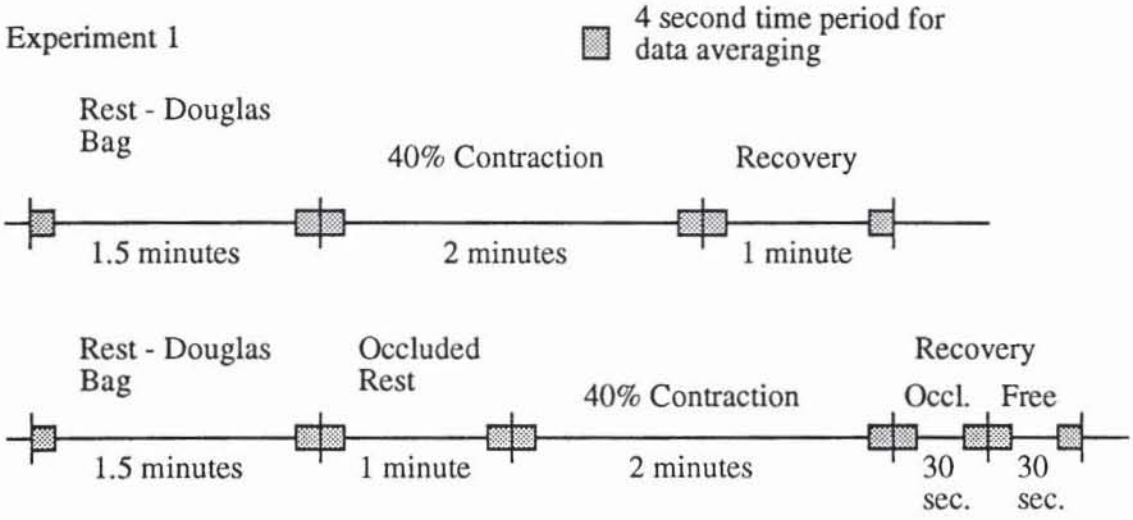
Expt. 2: Cytochrome Oxidase (CtOx)

As already mentioned, using the larger optodes gave slightly steadier signals from the near infrared spectrophotometer. Despite this, no significant redox change was seen in the CtOx oxidation state in the non-occluded bouts of isometric grip (Fig. 4.19). In the occluded bout at 30% MVC however, the CtOx became more oxidised over the 2 minutes of exercise at a rate of 0.5 ± 0.2 $\mu mol.min^{-1}$, significantly greater than that seen during the 20% MVC bouts (0.1 ± 0.1 $\mu mol.min^{-1}$). Once more there was no significant change in the cytochrome oxidase oxidation state during the first 30 seconds and the last 1.5 minutes of exercise (Fig. 4.20) until the final occluded bout. Here, CtOx became significantly oxidised in both periods, increasing at a significantly greater rate over the final 1.5 minutes (0.4 ± 0.2 $\mu mol.min^{-1}$) than during the final 1.5 minutes of the initial 20% non-occluded bout (0.1 ± 0.1 $\mu mol.min^{-1}$). In the minute of recovery following exercise, all the CtOx redox states became more oxidised, showing positive (further) shifts, but there was no significant variation with exercise intensity (Fig. 4.21).

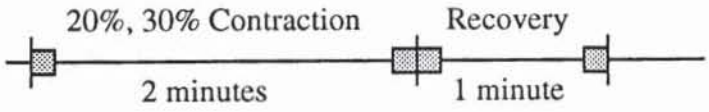
Expt. 2: Blood Lactate

Blood from the antecubital vein of the exercising arm was sampled for lactate concentration initially 30 seconds after the cessation of contraction, and then every minute thereafter until the lactate concentration had peaked. In each subject, the maximum lactate concentration was obtained in the second or third blood sample, 1.5 - 2.5 minutes after the end of handgrip contraction. The difference between this and the resting blood lactate concentration determined before each bout of exercise was used in the analysis and plotted in Fig. 4.22. Quite small increases in venous lactate concentration (1.2 - 1.8 $mmol.l^{-1}$) were induced by isometric exercise, and it is interesting that the cuff made no difference, when applied to the 30% MVC (1.75 ± 0.43 $mmol.l^{-1}$ with free blood flow, compared to 1.78 ± 0.39 $mmol.l^{-1}$ again under occlusion).

Fig. 4.1



Experiment 2



Analysis during 2 minute contraction:

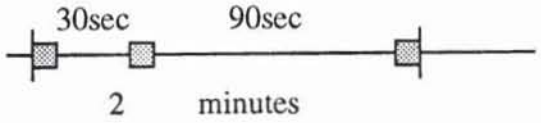


Fig. 4.1
Analysis of NIRS data.

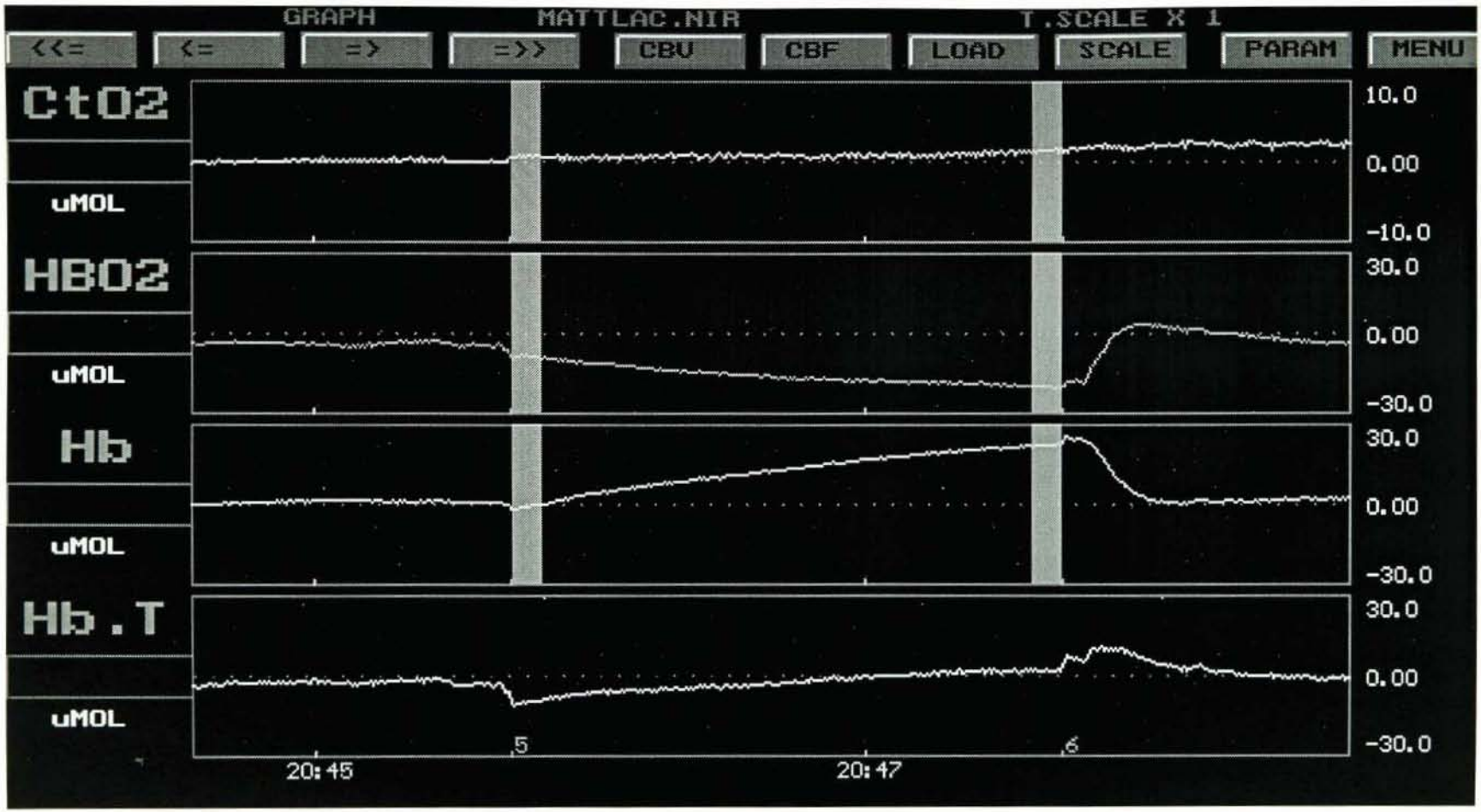


Fig. 4.2: Trace of 2 minute period of isometric grip contraction at 30% MVC under brachial cuff occlusion, showing the two 4-second periods of analysis at the beginning and end of exercise. Headings on the left refer to oxidised cytochrome oxidase (CtO2), Oxyhaemoglobin (HbO2), Deoxyhaemoglobin (Hb), and Total Haemoglobin (Hb.T [= HbO2 + Hb]). Values on the right are full scale deflection concentration changes in mM, from zero at rest.

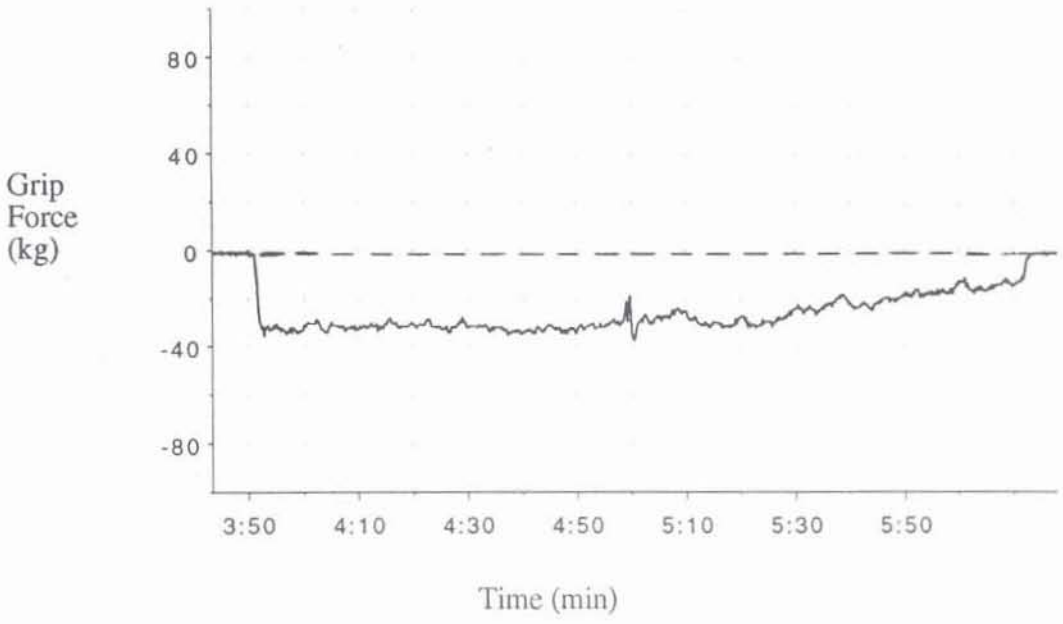


Fig. 4.3

Example of a handgrip force trace over 2 minutes of occluded isometric exercise at 40% MVC.

Fig. 4.4

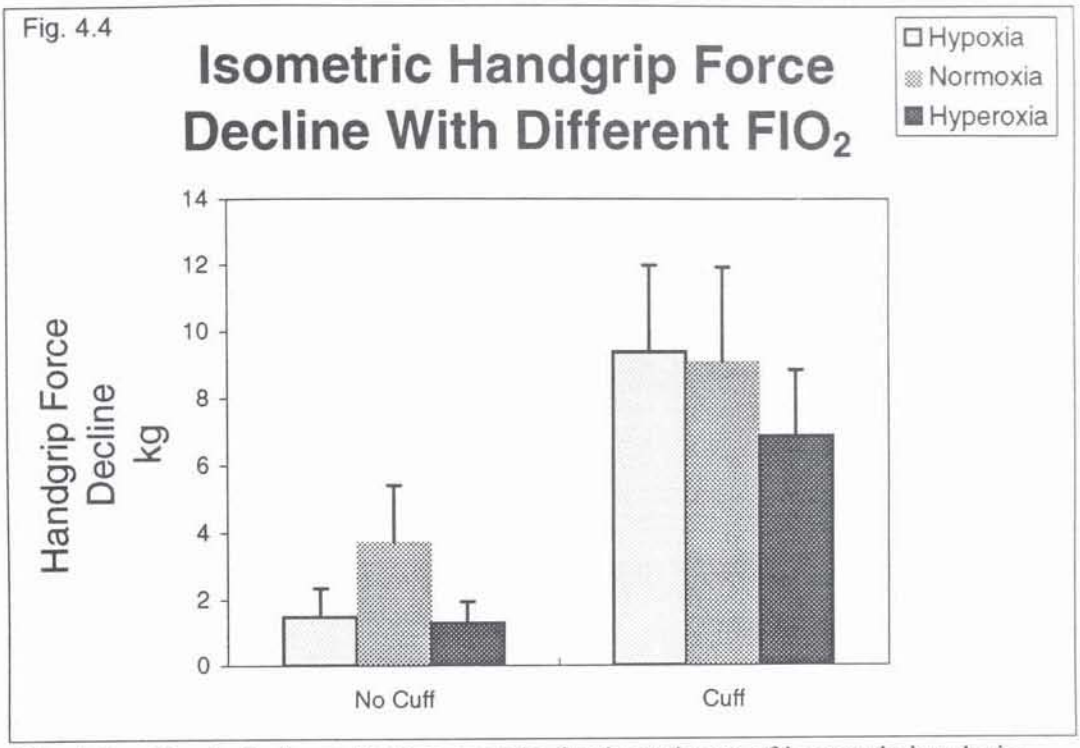


Fig. 4.4: Handgrip force decline over the 2 minute bouts of isometric handgrip exercise at 40% MVC, with free forearm blood flow or under brachial cuff occlusion, whilst breathing hypoxic, normoxic, or hyperoxic gas mixtures.

Fig. 4.5

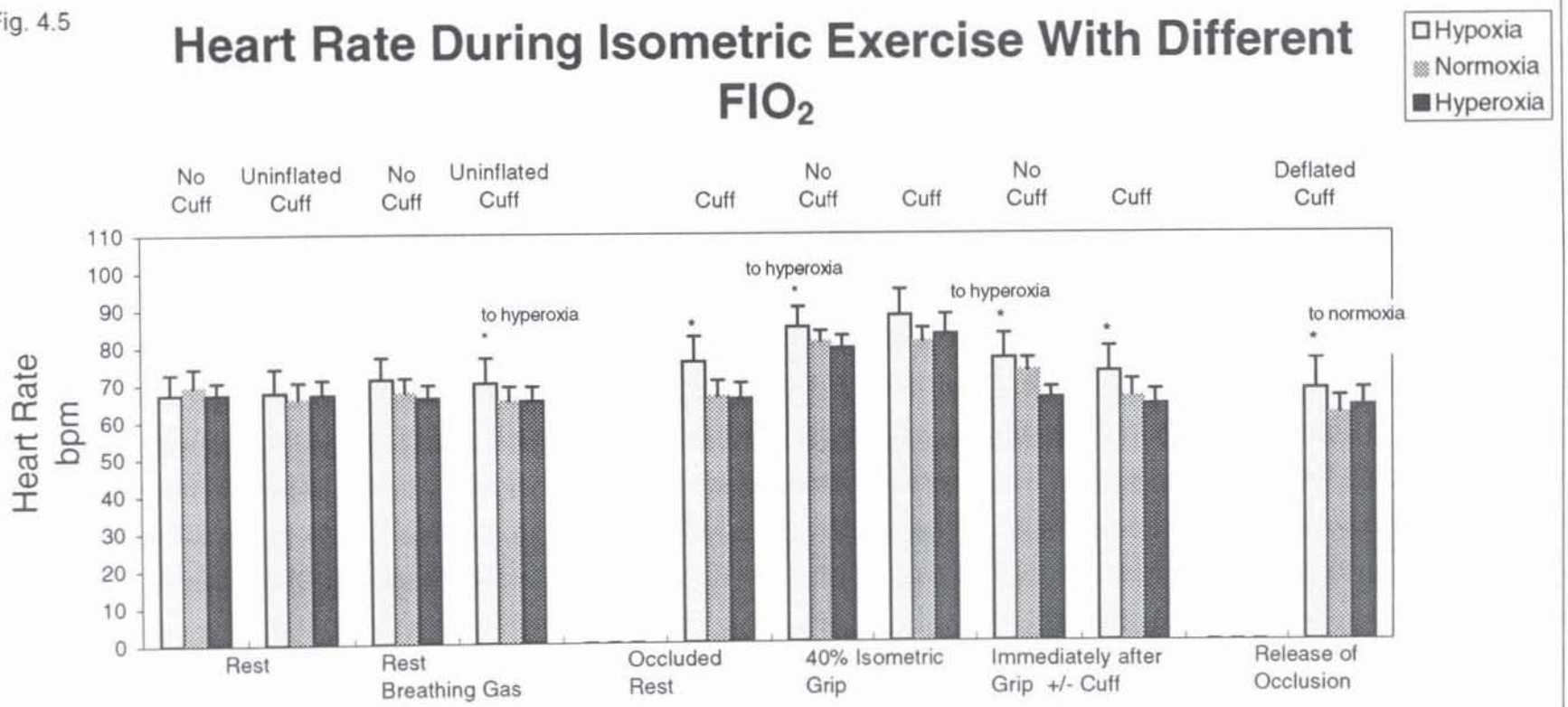


Fig. 4.5: Heart rate over the different stages of the isometric handgrip exercise protocol, with free forearm blood flow or under brachial occlusion, whilst breathing hypoxic, normoxic, or hyperoxic gas mixtures.

Fig. 4.6

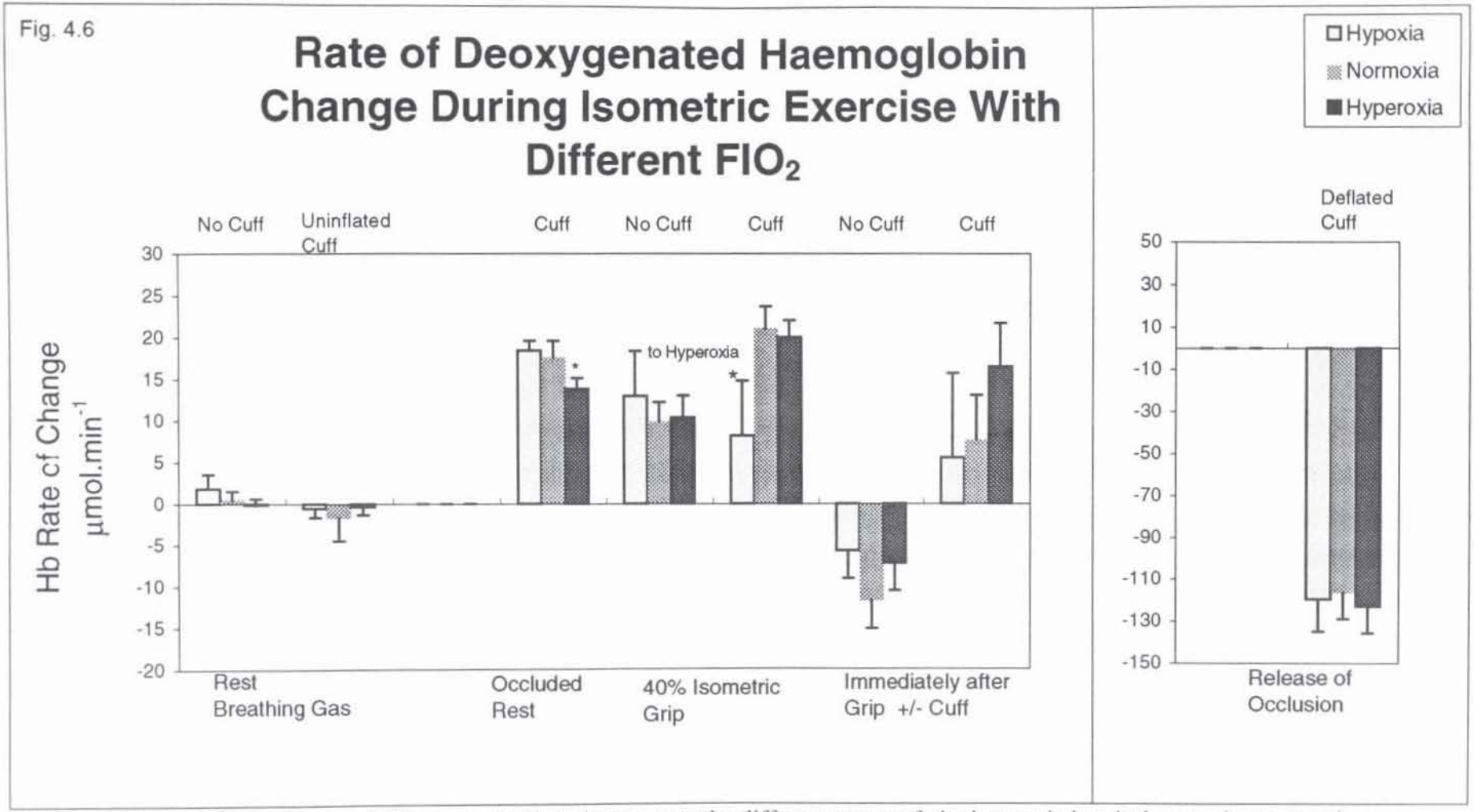


Fig. 4.6: Rates of Deoxyhaemoglobin concentration change over the different stages of the isometric handgrip exercise protocol, with free forearm blood flow or under brachial occlusion, whilst breathing hypoxic, normoxic, or hyperoxic gas mixtures. Right panel redrawn to different scale.

Fig. 4.7

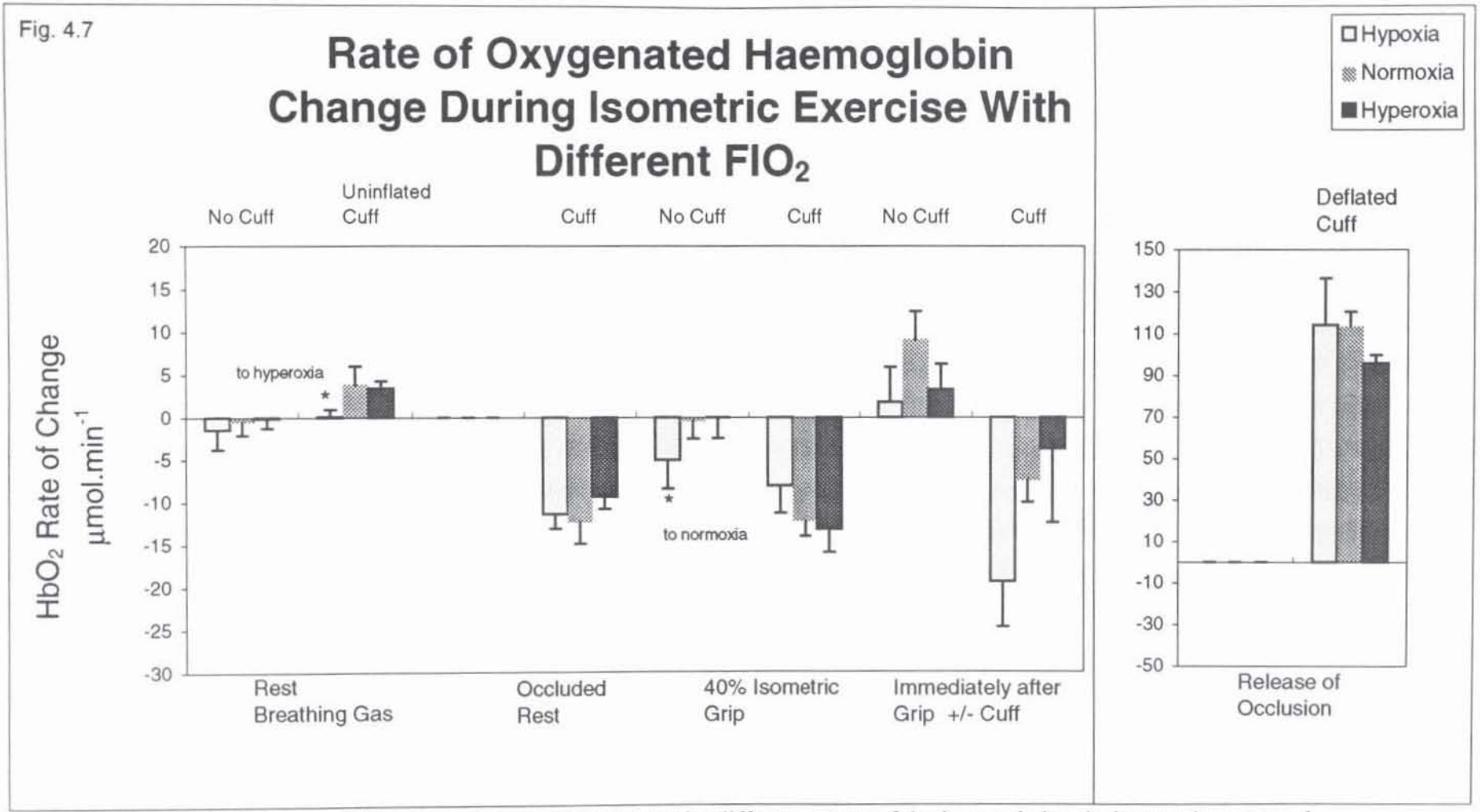


Fig. 4.7: Rates of Oxyhaemoglobin concentration change over the different stages of the isometric handgrip exercise protocol, with free forearm blood flow or under brachial occlusion, whilst breathing hypoxic, normoxic, and hyperoxic gas mixtures. Right panel redrawn to different scale.

Fig. 4.8

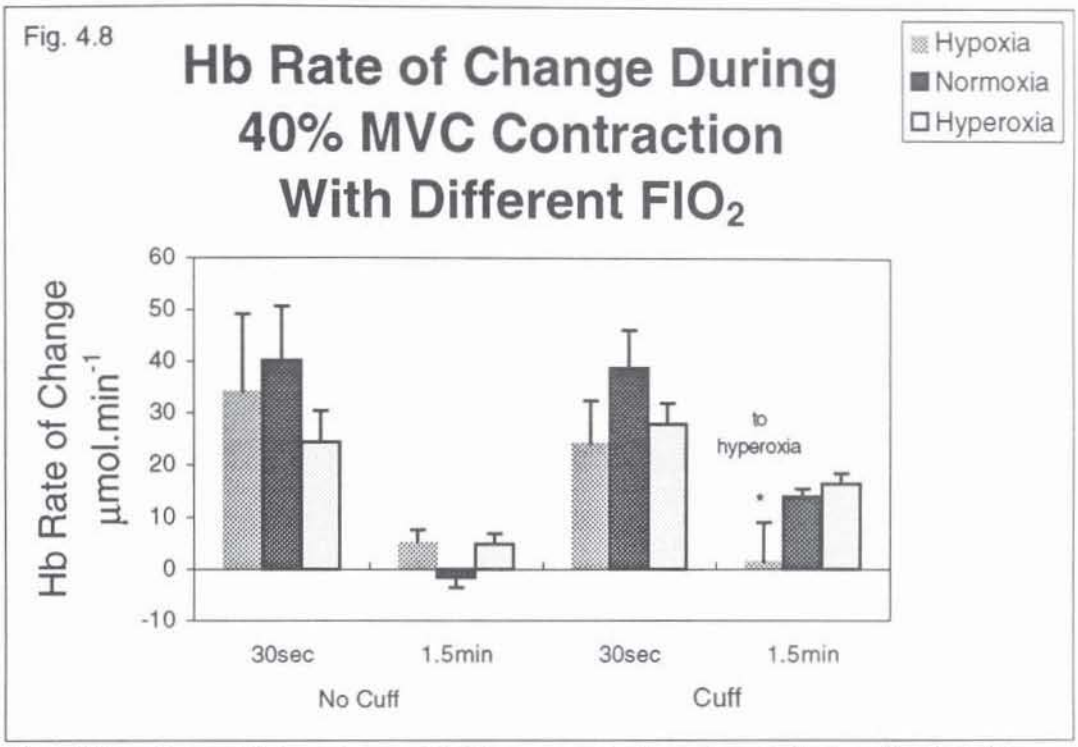


Fig. 4.8: Rates of Deoxyhaemoglobin concentration change during the first 30 seconds and the subsequent 1.5 minutes, of the 2 minute isometric handgrip contraction at 40% MVC with free forearm blood flow or under brachial occlusion, whilst breathing hypoxic, normoxic, and hyperoxic gas mixtures.

Fig. 4.9

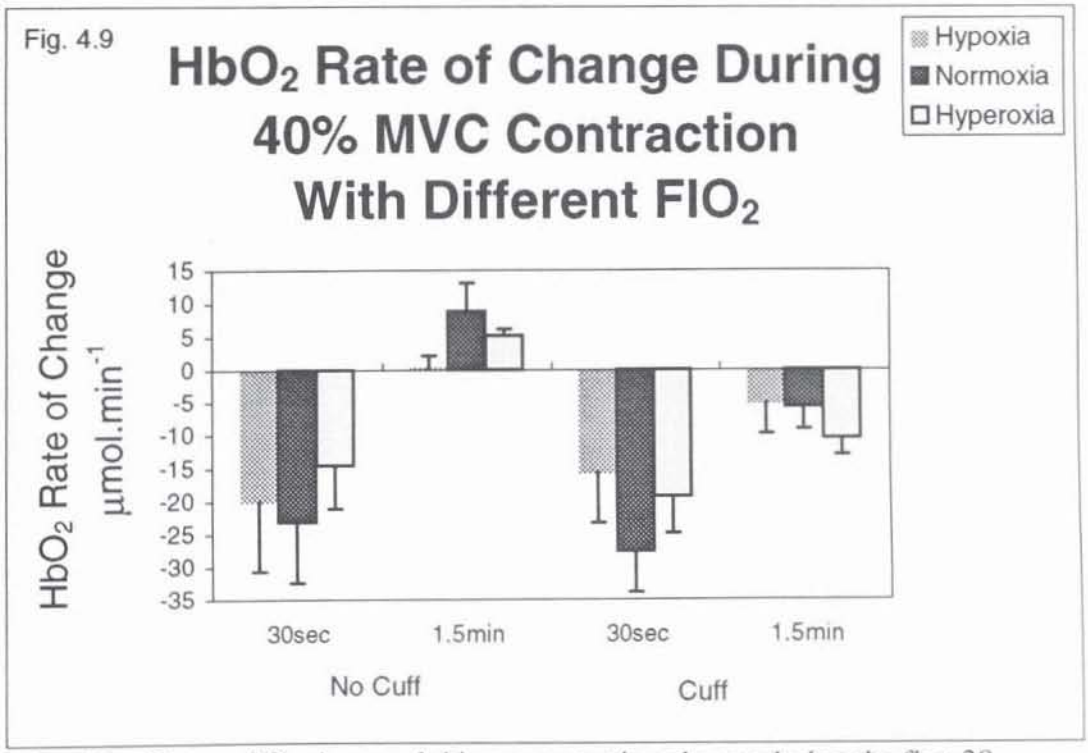


Fig. 4.9: Rates of Oxyhaemoglobin concentration change during the first 30 seconds and the subsequent 1.5 minutes, of the 2 minute isometric handgrip contraction at 40% MVC with free forearm blood flow or under brachial occlusion, whilst breathing hypoxic, normoxic, and hyperoxic gas mixtures.

Fig. 4.10

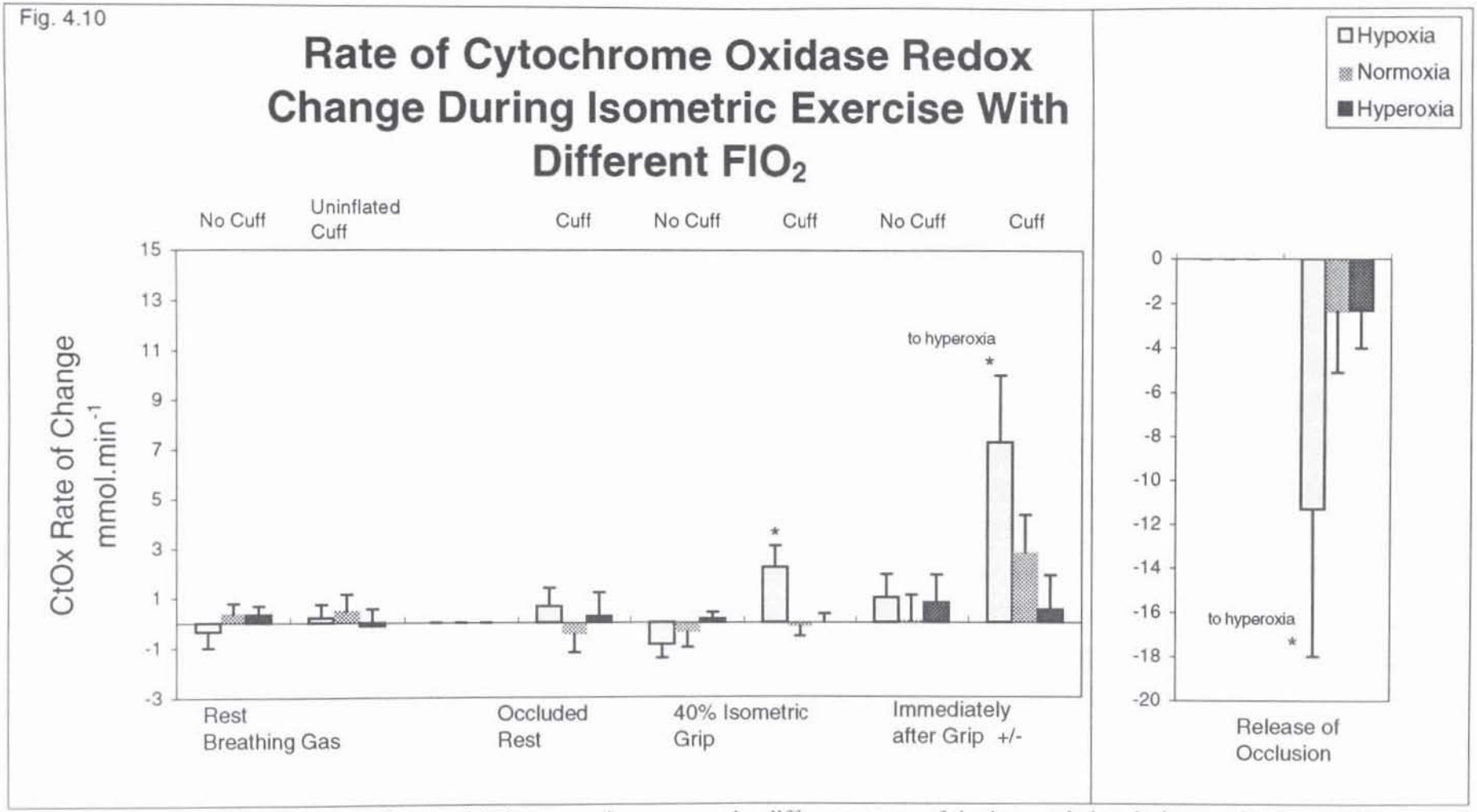


Fig. 4.10: Rates of Cytochrome Oxidase oxidation state change over the different stages of the isometric handgrip exercise protocol, with free forearm blood flow or under brachial occlusion, whilst breathing hypoxic, normoxic, and hyperoxic gas mixtures. Right panel redrawn to different scale.

Fig. 4.11

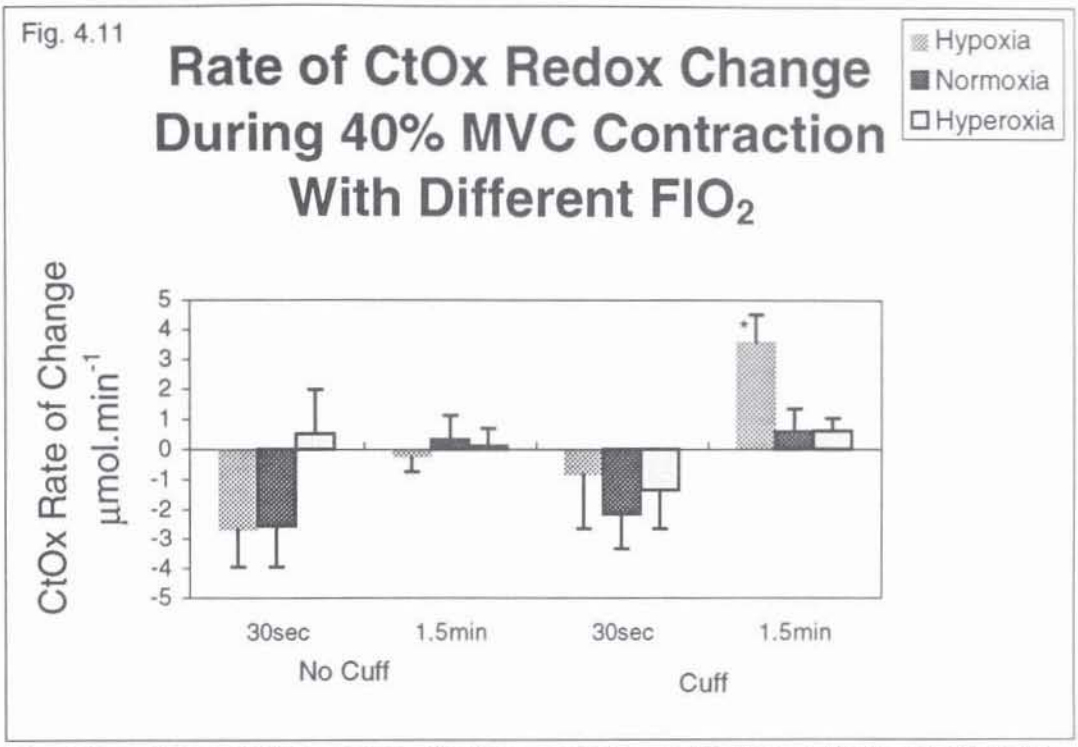


Fig. 4.11: Rates of Cytochrome Oxidase oxidation state change during the first 30 seconds and the subsequent 1.5 minutes, of the 2 minute isometric handgrip contraction at 40% MVC, with free forearm blood flow or under brachial occlusion, whilst breathing hypoxic, normoxic, and hyperoxic gas mixtures.

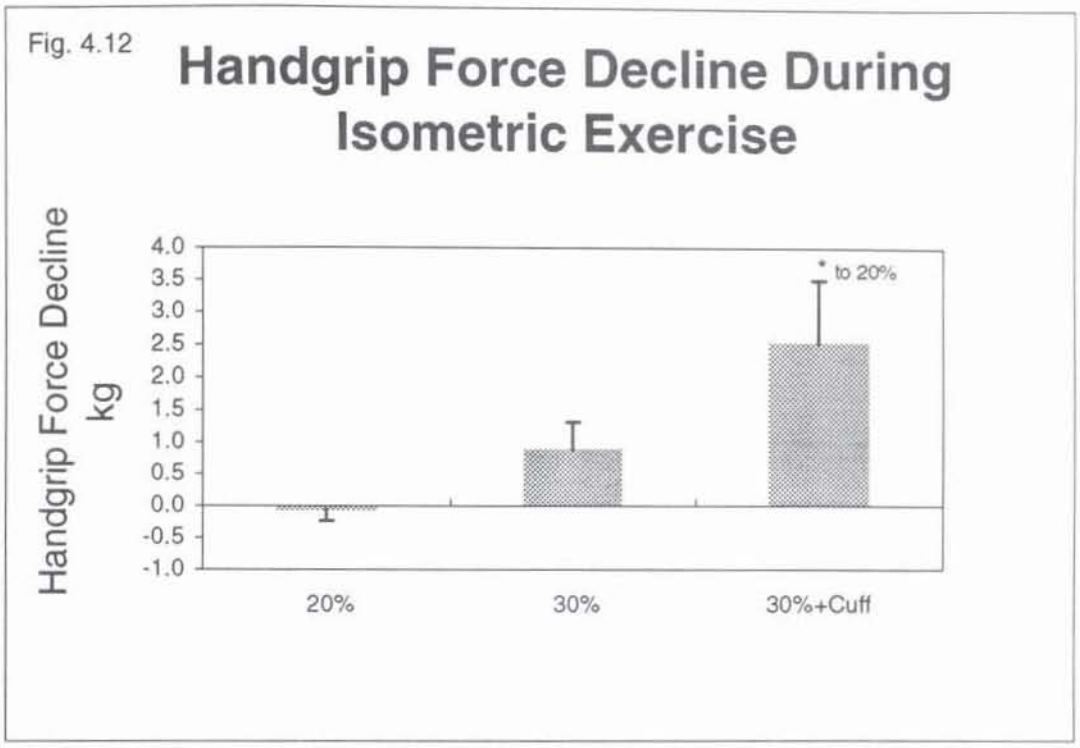


Fig. 4.12: Handgrip force decline over 2 minute bouts of isometric handgrip exercise at 20% MVC and 30% MVC with free forearm blood flow, or 30% MVC again under brachial occlusion.

Fig. 4.13

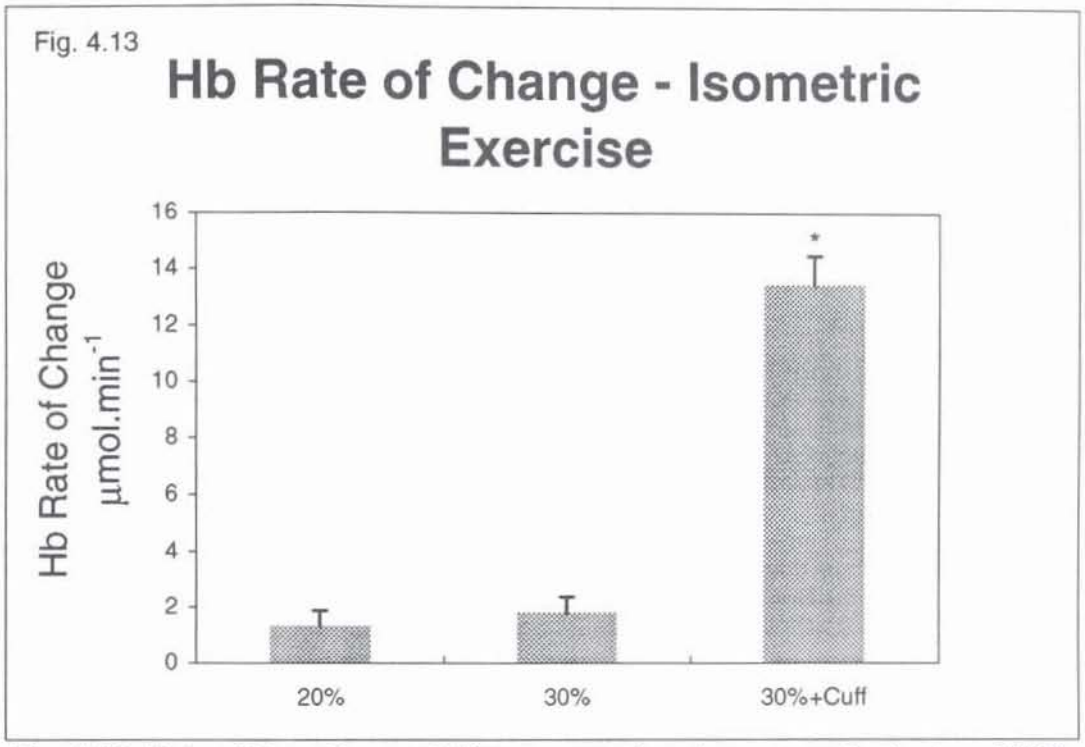


Fig. 4.13: Rate of Deoxyhaemoglobin concentration change over 2 minutes of 20% and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

Fig. 4.14

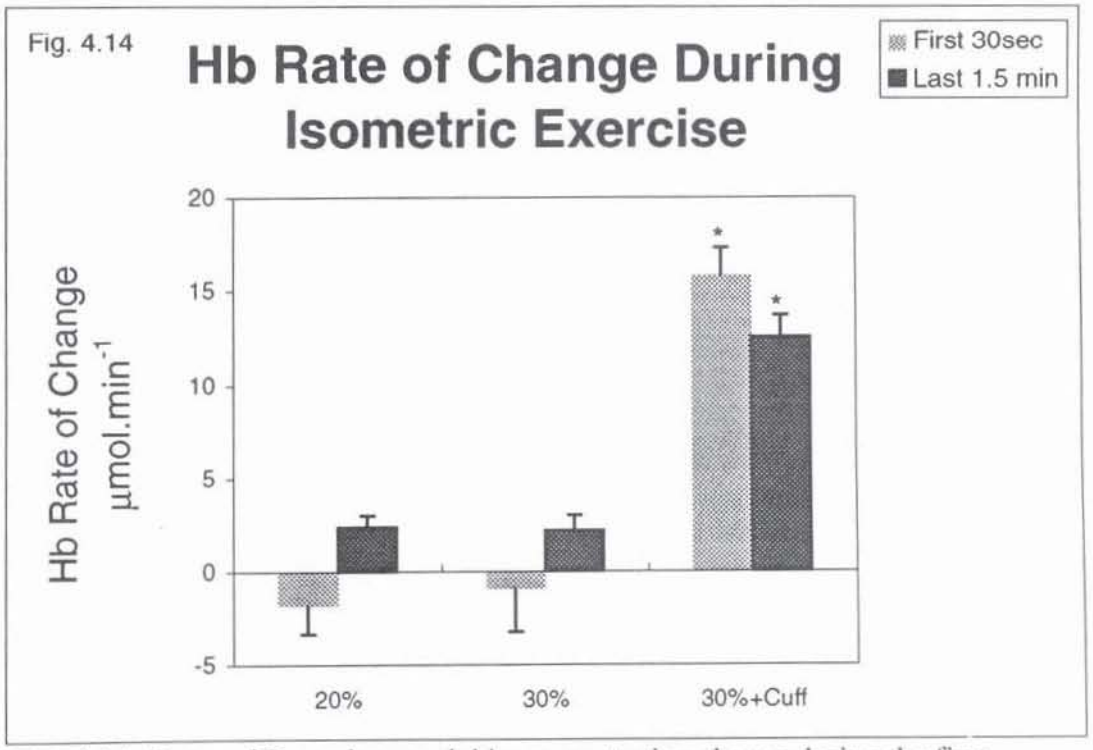


Fig. 4.14: Rates of Deoxyhaemoglobin concentration change during the first 30 seconds and the subsequent 1.5 minutes, of the 2 minute isometric handgrip contraction at 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

Fig. 4.15

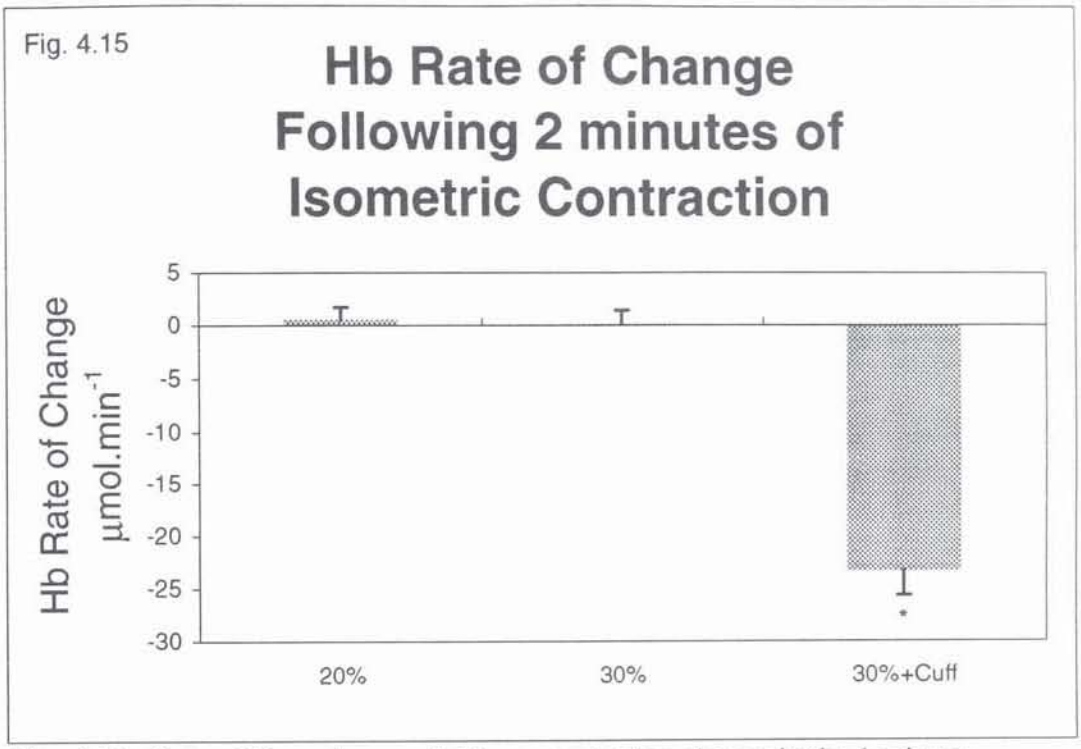


Fig. 4.15: Rate of Deoxyhaemoglobin concentration change in the 1 minute following isometric contraction for 2 minutes at 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

Fig. 4.16

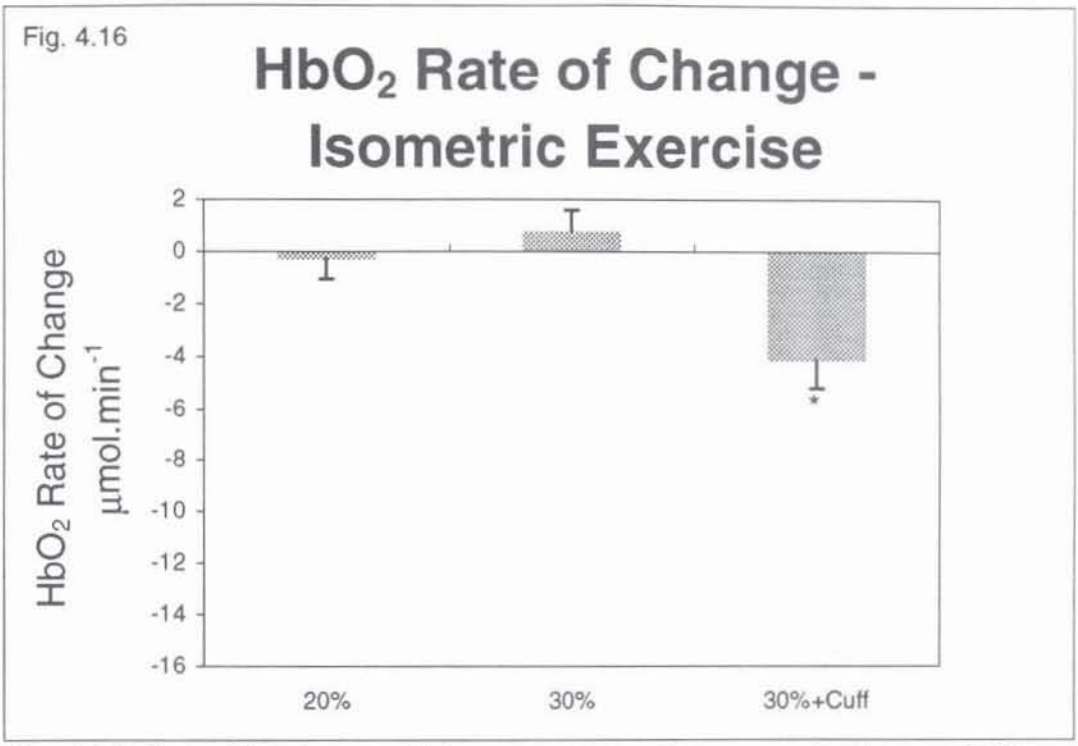


Fig. 4.16: Rate of Oxyhaemoglobin concentration change over 2 minutes of 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

Fig. 4.17

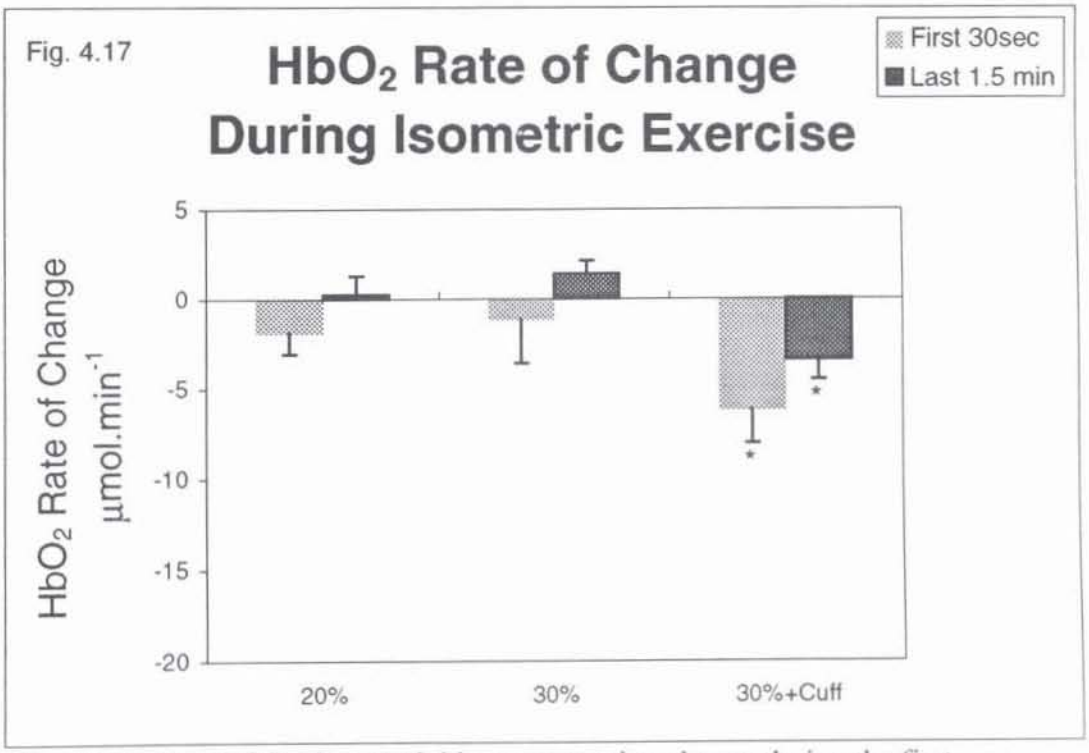


Fig. 4.17: Rates of Oxyhaemoglobin concentration change during the first 30 seconds and the subsequent 1.5 minutes, of the 2 minute isometric handgrip contraction at 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

Fig. 4.18

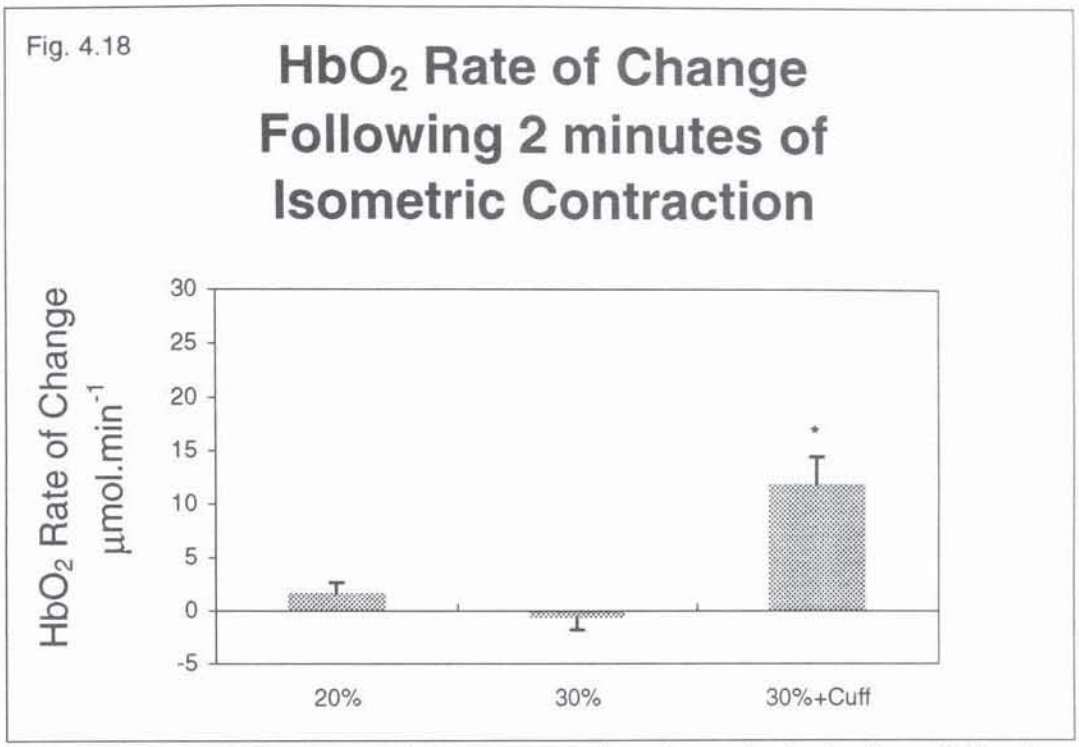


Fig. 4.18: Rate of Oxyhaemoglobin concentration change in the 1 minute following isometric contraction for 2 minutes at 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

Fig. 4.19

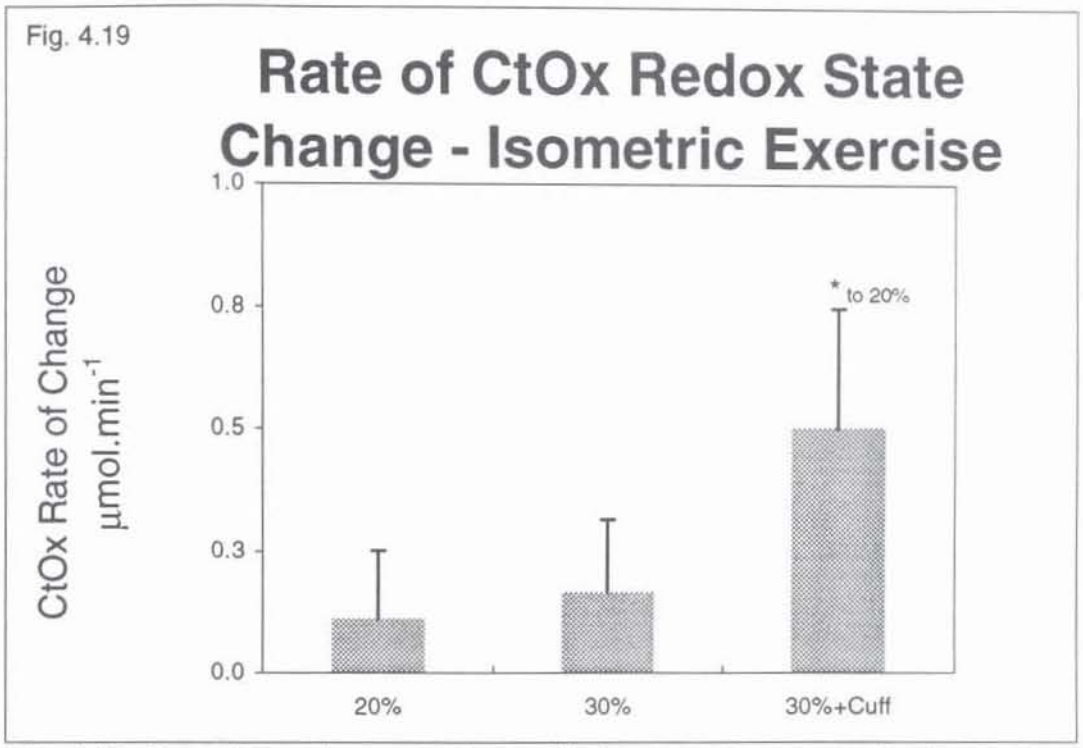


Fig. 4.19: Rate of Cytochrome Oxidase oxidation state change over 2 minutes of 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

Fig. 4.20

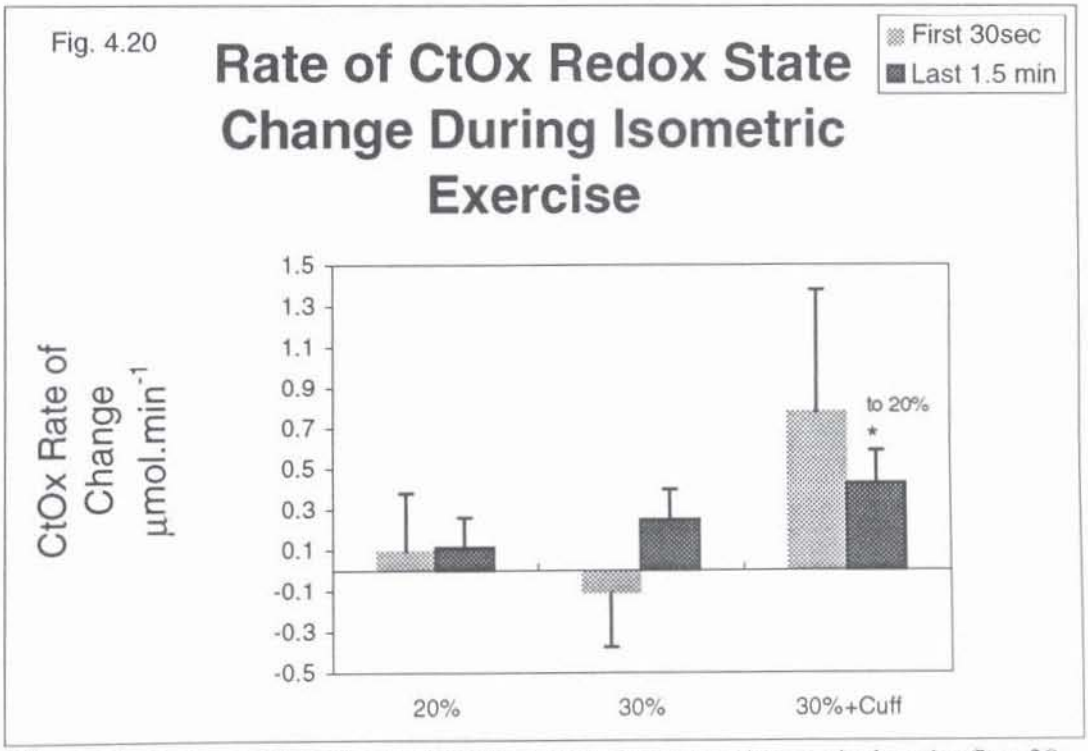


Fig. 4.20: Rates of Cytochrome Oxidase oxidation state change during the first 30 seconds and the subsequent 1.5 minutes, of the 2 minute isometric handgrip contraction at 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again brachial occlusion.

Fig. 4.21

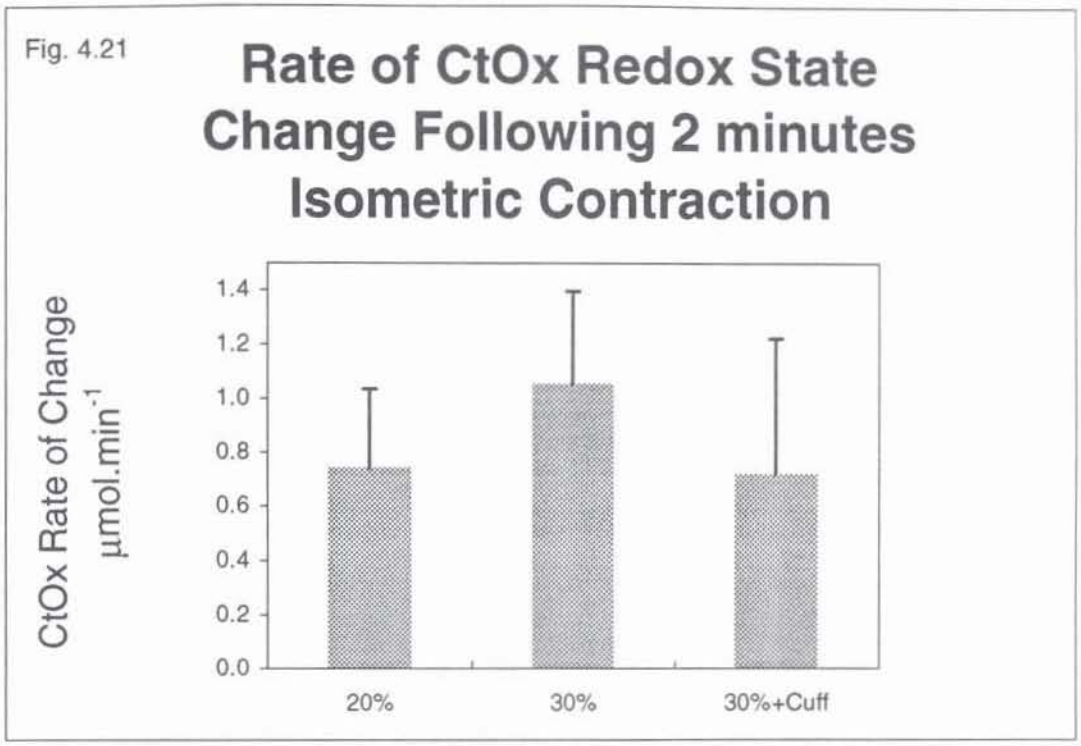


Fig. 4.21: Rate of Cytochrome oxidase oxidation state change in the 1 minute following isometric contraction for 2 minutes at 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

Fig. 4.22

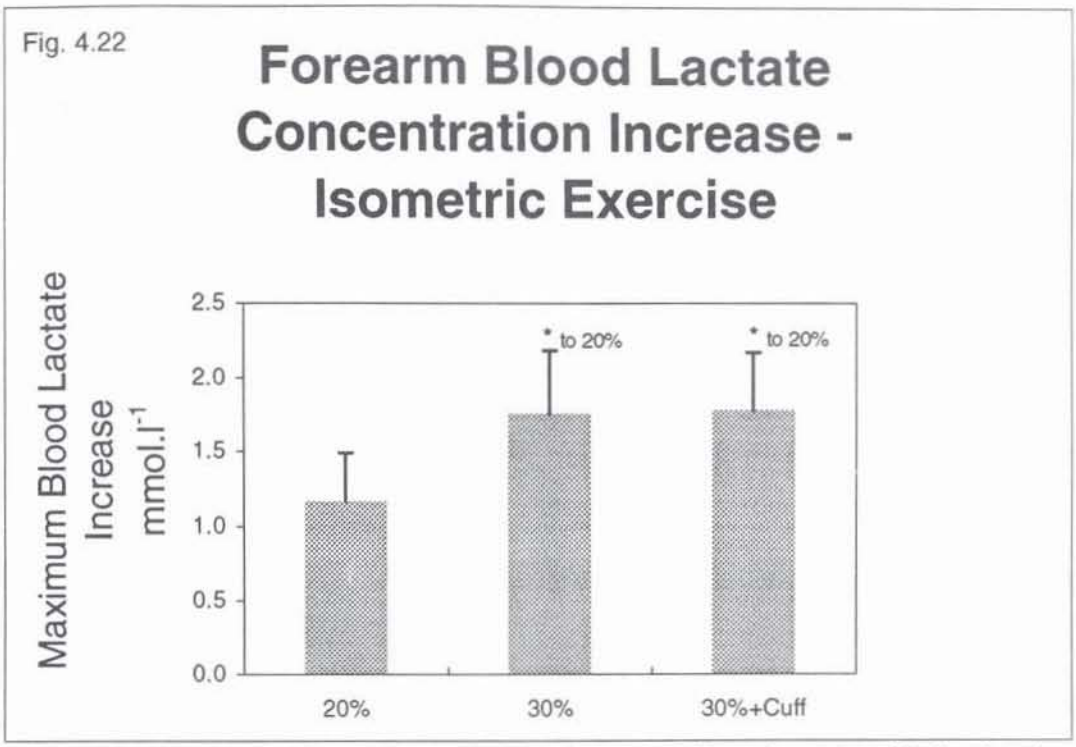


Fig. 4.22: Maximum lactate change in blood sampled from the antecubital vein of the forearm after 2 minutes of isometric exercise at 20% MVC and 30% MVC with free forearm blood flow, and 30% MVC again under brachial occlusion.

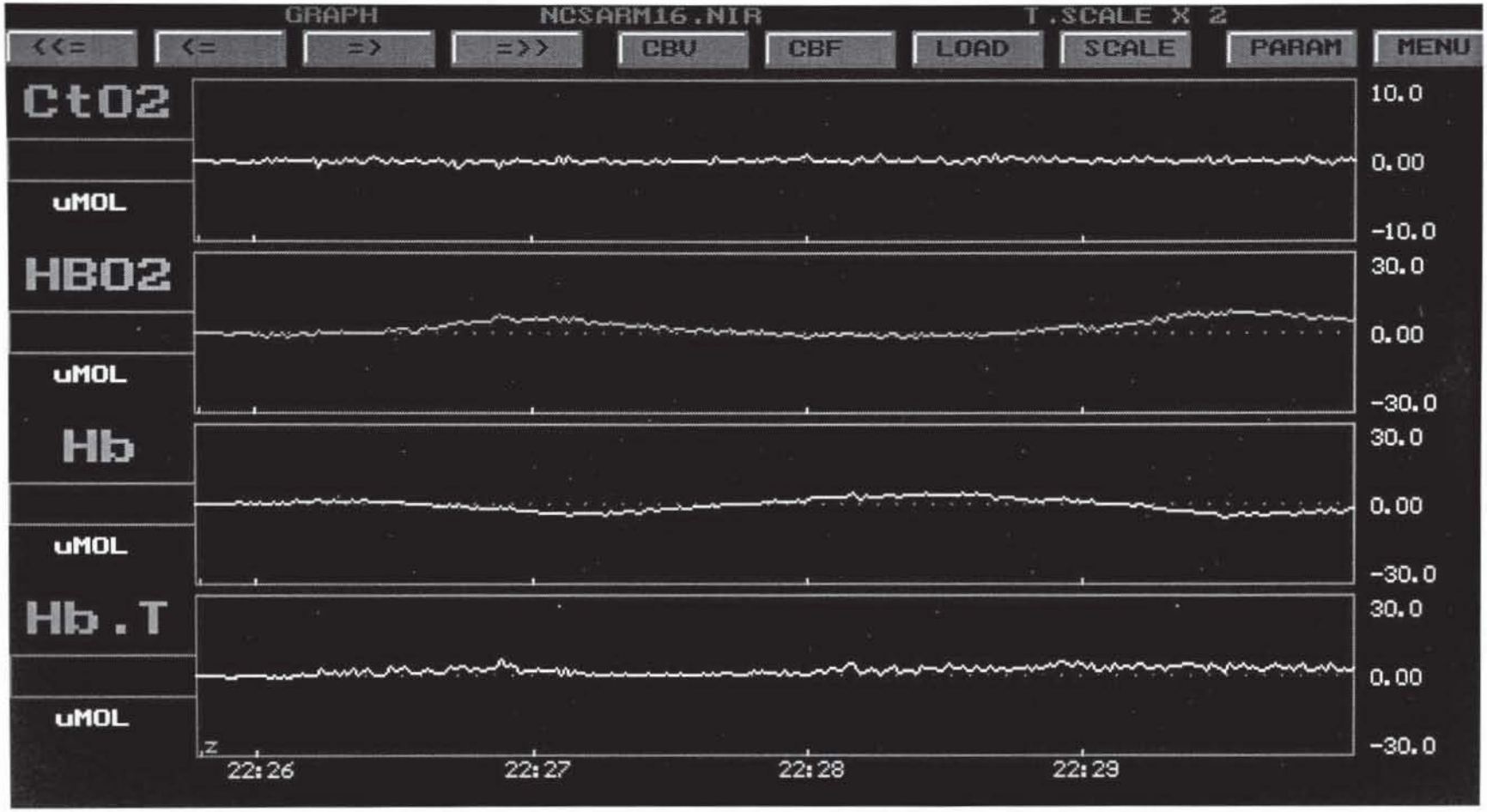


Fig. 4.23: Trace showing rhythmic fluctuations of HbO2 and Hb at rest. Headings on the left refer to oxidised cytochrome oxidase (CtO2), Oxyhaemoglobin (HbO2), Deoxyhaemoglobin (Hb), and Total Haemoglobin (Hb.T [= HbO2 + Hb]). Values on the right are full scale deflection changes in mM, from zero at rest.

DISCUSSION

As we saw in Chapters 1 and 2, in near infrared spectroscopy (NIRS) of tissue, light attenuation is due to:

- absorption by chromophores of variable concentration (deoxyhaemoglobin, oxyhaemoglobin, oxidised cytochrome oxidase),
- absorption by chromophores of fixed concentration (e.g. melanin in the skin, bone),
- light scatter (e.g. due to light travelling through regions of mismatched refractive indices).

NIRS is usually concerned with trying to monitor the first category - changes in chromophores of variable concentration.

Heart Rate

Heart rate was only recorded in Experiment 1, when isometric handgrip contractions were performed in conditions of differing FIO_2. In the present study, the hypoxic conditions tended to produce significantly higher heart rate values than the normoxic and hyperoxic conditions (Fig. 4.5, Table 4.1). The heart rate responds to changing blood gas concentrations sensed through the peripheral and central chemoreceptors (Jennett, 1989; Berne & Levy, 1990). Moderate degrees

of hypoxia characteristically increase heart rate, by increasing sympathetic cardioaccelerator nervous activity through the peripheral chemoreceptors, depressing the medullary vagal centre. Heart rate changes tend to be secondary to ventilatory effects. The hyperoxic conditions tended to produce a general, though non-significant, reduction in heart rate compared to normoxia. Breathing 100% O_2 will produce a prompt, brief depression of ventilation and heart rate. The raised oxygen tension reduces the activity and sensitivity of the peripheral chemoreceptors, blunting any further response to changing arterial CO_2 concentration, or arterial pH. However, the reduction in heart rate and ventilation is only transient, as the central chemoreceptors eventually correct for the resulting increase in arterial PCO_2.

Handgrip Force

Some decline of handgrip force was seen in both studies. The general pattern was of greater force decline at higher intensities of isometric exercise (Fig. 4.12) and particularly in cuff-occluded conditions (Figs. 4.4, 4.12). There was however, large individual subject variation. The ability to generate force was almost completely lost in some subjects in the occluded 40% bout, but quite well maintained by others. Force loss was more moderate in the 30% study, but was still significant in the occluded bouts. The force loss in the 40% study showed no significant relation to the gas mixture breathed, although during the occluded bouts of exercise, the hypoxic conditions produced slightly greater force decline.

However, in the initial, non-occluded bouts at 40% MVC, the normoxic condition actually produced the greatest force decline. This result perhaps seems strange, but may be due to a learning effect, as the random nature of the allocation of breathing mixture put normoxia first in 5 of the 8 subjects.

Deoxyhaemoglobin, Oxyhaemoglobin

Prior to exercise, and in the non-occluded bouts of exercise at 20% and 30% MVC, very little change was seen in the Hb and HbO_2 concentrations (Figs. 4.6, 4.7, 4.13, 4.16). These exercise intensities were quite moderate, and coupled with the general low energy cost of isometric exercise (Asmussen, 1981) enabled the exercise to be carried out without a large oxygen demand and corresponding change in haemoglobin oxygenation. At 40% MVC however, a significant increase in Hb is seen (Fig. 4.6). The subjects found this intensity quite severe. It was noted in the introduction to this chapter that at intensities of 20% and 30% MVC, arm-muscle blood flow increases during exercise, and then increases further on cessation of contraction (Lind & McNichol, 1967). It seems that at these intensities, the blood flow is unable to adapt sufficiently to meet the metabolic requirements of the exercising muscle, and clear accumulating vasodilators sufficiently (such as H^+, CO_2, K^+, adenosine etc.) until the end of exercise, whereupon the 'blood flow debt' is paid off. At forces of 40% MVC and more, there will be a decrease in muscle blood flow during exercise. The increased energy demand from the higher 40% MVC intensity, coupled with greater partial

occlusion of the muscle blood flow from the elevated intramuscular pressure, would cause more Hb to be produced and held for some time in the muscle.

There was a slightly greater increase in Hb in the cuff-occluded 40% bout compared to the unoccluded bout. The fact that much the same change in the Hb concentration is seen in both the cuff-free and cuff-occluded 40% exercises is consistent with the oxygen demand of the two 40% isometric contraction bouts being equal. The HbO_2 concentration however only fell significantly when the cuff was inflated.

The Hb and HbO_2 values did not change by equal and opposite amounts during exercise (Figs. 4.2, 4.6, 4.7). The slightly greater increase in Hb accumulation compared to the reduction of the HbO_2 values was presumably caused by net blood flow into the volume monitored by the NIR light. Hb can only be formed from HbO_2 in regions of the muscle requiring oxygen. In the region monitored, all Hb formed will therefore come from HbO_2, causing an increase in the rate of Hb generation and an equal decrease in HbO_2. However, any increase in blood flow (and therefore HbO_2) to the region of the muscle monitored by the optodes, will increase the HbO_2 concentration, and offset the decrease due to Hb generation.

In the non-occluded 40% exercise, this was very evident. The increase in Hb concentration was accompanied by a very much smaller decrease in the HbO_2 concentration. The low oxygen demand, and therefore low rate of HbO_2 reduction was adequately compensated for by an increase in blood flow. Hence only a minor

rate of HbO_2 fall was evident. The application of cuff-occlusion caused more equal changes in Hb and HbO_2. The occluded 40% exercise caused a larger rate of increase in the Hb concentration (Fig. 4.6) but a much greater relative change in HbO_2, the quantity of which now fell significantly over the exercise period (Fig. 4.7). The total stoppage of blood flow in the occluded condition will cause a greater decrease in HbO_2 compared to the non-occluded condition. This decrease in the HbO_2 concentration was however still not equal to the Hb increase in the cuff-occluded exercise. This suggests some blood re-distribution was still occurring within the vasculature of the occluded limb.

Exercise at 30% MVC will still allow some increase in muscle blood flow during isometric exercise, whereas during 40% MVC, exercise may cause a reduced blood flow within the exercising muscle. Therefore there will be a greater possibility of blood flow redistribution within an occluded muscle contracting at 30% MVC, than 40% MVC. This is supported by the results of the cuff-occluded 30% and 40% exercise bouts. The cuff-occluded 30% exercise caused a 13.4 $\mu mol.min^{-1}$ increase in Hb (Fig. 4.15), but only a 4.2 $\mu mol.min^{-1}$ decrease in HbO_2 (Fig. 4.12). The cuff-occluded 40% MVC showed an average of 16.4 $\mu mol.min^{-1}$ increase in Hb concentration, and an 11.2 $\mu mol.min^{-1}$ decrease in HbO_2 concentration. These figures suggest HbO_2 inflow to the area monitored by the optodes of 9.2 $\mu mol.min^{-1}$ at 30% MVC but only 5.2 $\mu mol.min^{-1}$ at 40%.

The sources of these blood inflows must be considered with caution. The possibility of leakage under the occluding cuff can not be excluded in the present

study. Leakage from under a 240 mmHg brachial cuff (as applied to the 40% contractions) is less likely than from under a 200 mmHg cuff (30% contractions). The reduction in the occlusion pressure was due to complaints of pain under the cuff due to the 240 mmHg pressure in the 40% study. Isometric exercise causes a greater increase in blood pressure than dynamic exercise, due to the increase in vasculature peripheral resistance (Lind & McNichol, 1967). Blood pressures of over 200 mmHg may have occurred in some subjects, possibly allowing some perfusion of the forearm muscles despite the brachial cuff.

The Hb and HbO_2 traces in some subjects seemed quite unstable, and would fluctuate during the rest periods between exercise bouts. This caused some variation in the recovery time while attempting to wait for stability before proceeding. The two signals were however, seen to fluctuate in equal, but opposite patterns (Fig. 4.24). In resting skeletal muscle, only a fraction of the total capillaries are open at any moment (Åstrand & Rodahl, 1986b). The individual capillaries do not stay constantly open or closed however, but alternate between the two conditions. This will cause periods of Hb production, coupled by a more or less equal decrease in HbO_2 concentration, when the capillaries within the area of muscle monitored by the optodes are predominantly closed. When the capillaries reopen, the opposite change will occur, and Hb will fall and HbO_2 increase due to the fresh supply of oxygenated blood. It seems likely that such periodic flow changes were occurring in the microvasculature under the optodes during the declining stages of post-exercise hyperaemia in the present experiments.

Cytochrome Oxidase Oxidation State

The cytochrome oxidase oxidation state showed no significant change during the non-occluded bouts of isometric exercise at any of the intensities used. During exercise under cuff-occlusion, however, the NIRS signal indicated that cytochrome oxidase became more oxidised during the course of exercise (Figs. 4.10, 4.19). The occluded bout at 30% MVC showed a slightly greater increase in oxidised cytochrome oxidase than the same exercise without occlusion, which had shown no significant redox change. The most severe conditions of forearm exercise used in this study, 40% MVC under cuff-occlusion, whilst breathing a 12% hypoxic gas mixture, produced a condition in which exercise elicited a marked increase in the cytochrome oxidase oxidation state (Fig. 4.10). In all conditions in which the enzyme became significantly more oxidised, the majority of change occurred in the last 1.5 minutes of the 2 minute bout (Figs. 4.11, 4.20).

Validation of the NIRS signal

The principles and general validation of the use of NIRS to monitor cytochrome oxidase redox state were discussed in the Introduction, on page 25. However, given the *prima facie* improbability of the results just discussed, it seems appropriate to recall the main points. The concentration of cytochrome oxidase in skeletal muscle is at least an order of magnitude lower than that of haemoglobin (Van Kuilenberg *et al.*, 1991), so its oxidation state is a much more difficult

parameter to study. The findings of Cooper *et al.* (1994) and Mancini *et al.* (1994) justify acceptance of changes in NIR absorbance by Hb and HbO_2 as accurate and representative of real physiological changes. The validity of the CtOx signal to be representative of real physiological changes in the oxidation state of cytochrome oxidase has been questioned. The lower concentration of cytochrome oxidase compared to haemoglobin has raised the possibility of cross-talk onto the cytochrome oxidase signal from changes in haemoglobin. Any 'noise' in the NIR signal, due to light scattering, will also be more evident in the much smaller cytochrome oxidase signal.

In the presence of such noise, most changes only become clear on analysis of the subject group as a whole. By taking the average of a 4-second period of data at the beginning of the 2 minute period of exercise, and a 4-second period at the end of exercise (Figs. 4.1, 4.2), and calculating the change per minute (to allow for slight changes in exercise duration, e.g. from the 4 subjects over the 2 studies who didn't manage the 2 minute duration of exercise), small changes in the mean cytochrome oxidase oxidation state became apparent. These were not in the direction expected, if the thinking of the Wasserman laboratory (e.g. Wasserman & Koike, 1992) is correct, i.e. that the enzyme would become more reduced as exercise intensity increased. In the occluded bouts of isometric exercise, NIRS showed that the cytochrome oxidase enzyme became more oxidised.

Prior to 1991, the validity of the NIRS technique, and the instrumentation used had received minimal attention in the literature. However, Cope (1991) developed NIRS into a powerful, clinically acceptable technique. He took into account the absorption and scattering components of the NIR light in tissue, and developed the algorithm employed by the NIRO-500, which used 4 wavelengths of NIR light and the respective absorption spectra of Hb, HbO_2, and CtOx at each wavelength, to calculate the changes in concentrations of the three chromophores (Appendix 2).

The algorithm itself has been found to give results in broad agreement with those from a continuous wavelength charge-coupled device (CCD)-based tissue spectrophotometer (Matcher *et al.*, 1995). The technique of NIR spectroscopy has been tested both with respect to oxygenated and deoxygenated haemoglobin, and to cytochrome oxidase. Mancini *et al.* (1994) showed NIR absorption to be closely correlated with venous oxygen levels and haemoglobin saturation, with only minor contributions from myoglobin. Cytochrome oxidase, being a much smaller signal, will be much more susceptible to interference from other signals such as Hb and HbO_2. However, studies in which the blood of experimental animals has been replaced by a perfluorocarbon substitute (Wray *et al.*, 1988; Cope, 1991), and where the change in the cytochrome oxidase NIR signal has been compared in the presence of large haemoglobin oxygen saturation and concentration changes (Cooper *et al.*, 1994), have shown the technique and the algorithm used in this thesis to be accurate.

Mechanisms of Redox Change

The question therefore arises of a biochemical explanation for the unexpected direction of the cytochrome oxidase redox change indicated by the NIRS signals from occluded exercising forearms. Connett *et al.* (1986) found that oxygen never became limiting in dog gracilis, even in maximum, electrically stimulated dynamic exercise. These data do not disagree with that finding. Cytochrome oxidase becoming more reduced would reasonably be considered to be a sign of inadequate oxygen supply (Cooper *et al.*, 1994; Matcher *et al.*, 1995). Cytochrome oxidase becoming more oxidised over a period of exercise suggests either:

1) an improvement in previously inadequate O_2 supply, or
2) a reduction in the flux of electrons through the ETC enzyme complex (Cooper *et al.*, 1996; Cooper, 1996).

Of these two possibilities, (1) can of course be rejected, as there is no question of O_2 supply being inadequate in healthy, unoccluded muscles at rest - as they all were at the start of each experiment. While O_2 supply remains adequate, (2) would mean that the enzyme spent more of its time in an oxidised state awaiting an electron. The Km of cytochrome oxidase for O_2 has been quoted as less than 1 μM (Murphy & Brand, 1987). Once it received electrons and became reduced, with oxygen never being limiting the cytochrome oxidase can then be expected to donate its electrons to molecular oxygen, and reduce it to water. Cytochrome

oxidase has 4 redox active groups - two haem iron centres (haem a, and haem a_3), and two copper centres (Cu_A, and Cu_B - Elwell, 1995; Gennis & Ferguson-Miller, 1995). Electrons are first transferred from cytochrome c to the Cu_A redox centre of cytochrome oxidase, then to the haem a centre, and then on to the Cu_B and haem a_3 redox active groups. The oxygen binding site of the enzyme is the binuclear unit formed from the Cu_B and haem a_3 groups, which is where oxygen is reduced to form water. Cytochrome oxidase can therefore accept up to 4 electrons, 2 of which are used each time to reduce oxygen to water.

At rest, the enzyme complex will in theory be in an intermediate redox state, in one of five different stages of reduction (awaiting an electron, or with one, two, three, or all four electron accepting sites occupied - the last instance meaning that it is ready to reduce molecular oxygen). As only two electrons are donated to molecular oxygen from the haem a_3-Cu_B centre, in normal conditions the enzyme may only alternate between carrying two, three, or four electrons. Assuming oxygen to be plentiful, then as soon as the CtOx receives 4 electrons it will go on and lose 2 of the electrons reducing oxygen to water. The enzyme may then only effectively alternate between carrying two and three electrons, and be between 50% and 75% reduced at rest. Stainsby *et al.* (1989) however suggested that cytochrome oxidase was 50-80% oxidised at rest in an *in situ* dog gastrocnemius-plantaris preparation. Hampson & Piantadosi (1988) suggested that human muscle cytochrome oxidase was 67% oxidised at rest, and Hemple *et al.* (1977) quoted resting cat brain to be 15% oxidised at rest. The variation in redox estimate can possibly be attributed to the use of different techniques and tissues. Direct muscle

biochemical analysis has uncertainties associated with the extent of biochemical processes in the time between sampling and freezing (Wasserman *et al.*, 1986), and methods of cell surface fluorescence have problems of movement artefact and lack of discrimination between, for example, NADH and NADPH (Henriksson *et al.*, 1986). Stainsby *et al.* (1989) felt they had obtained reliable data for 100% reduced cytochrome oxidase by respiring with 100% N_2, but were unsure how near they were to the fully oxidised condition when breathing 100% O_2. This agrees with the theoretical picture sketched above. To obtain all four electron sites free of an electron may be more difficult to achieve than to obtain all the sites occupied by an electron.

One possible explanation for the changes in the cytochrome oxidase oxidation state seen in this thesis may be the environment of the enzymes of energy metabolism upstream from cytochrome oxidase. Conditions that stimulate lactate production can change the environment of the muscle, and the muscles' energy generating pathways. As illustrated from the study looking at lactate in the blood due to 2 minute bouts of 20% MVC, 30% MVC, and 30% MVC again under occlusion, lactate production increases as exercise intensity increases. At 30% MVC the blood flow in the muscle can still increase during exercise (Lind & McNichol, 1967), so the metabolites of muscle metabolism will not collect significantly in the muscle and are removed by the blood. No more lactate is released into the blood (and therefore one presumes no more is generated in the muscle) on application of the brachial cuff for the duration of the exercise. Considering this, one should first remember that the same amount of tension is

being maintained by the muscle, and isometric exercise is characterised by a relatively low energy cost anyway (Asmussen, 1981). In isometrically exercising muscle based on lactate release, it is estimated that 60% of the ATP produced is associated with anaerobic metabolism and lactate production (Henriksson *et al.*, 1986; Katz & Sahlin, 1988) compared to only 2% in dynamically exercising muscle (Sahlin *et al.*, 1987). The low metabolic demand is illustrated by only 1.75 $mmol.l^{-1}$ of lactate being produced by the forearm in 30% MVC exercise.

On inflation of the brachial cuff, the muscle essentially becomes a closed system. Metabolites generated by muscle metabolism will build up in the muscle fibre, in the immediate vicinity of where they are produced. This build up will change the intracellular environment from optimal energy generating conditions, and affect the energy generating enzymes and their pathways. The ability of the pathways to generate electrons will then by reduced, so less will be passed down the ETC to cytochrome oxidase. Fewer electrons will reach the terminal enzyme of the ETC, so the cytochrome oxidase will spend more time awaiting electrons, and become more oxidised overall.

An additional factor contributing to the changes seen in the cytochrome oxidase redox state may be associated with the effects of the mitochondrial environment on the mitochondrial membrane redox potential (Stainsby *et al.*, 1989) in turn affecting the flux of electrons through the cytochrome oxidase complex. Exercise increases the rate of extramitochondrial protons flowing into the mitochondrion through the ATP synthetase membrane channel, where they are required in the

regeneration of ATP from ADP. Reducing equivalents simultaneously generated from glycolysis and the citric acid cycle are transported down the electron transport chain, pumping protons back out of the mitochondrion to restore the membrane potential. Any change in the extramitochondrial proton concentration will affect the membrane potential, and may also effect the rate of electron pumping required to maintain the electron balance.

As mentioned above, isometrically exercising muscle is characterised by a greater anaerobic ATP production compared to dynamic exercise. In conditions of occluded isometric exercise, and particularly with the addition of hypoxia, protons generated from energy metabolism will accumulate in the extramitochondrial space. This will cause a reduction in the protons being pumped out of the mitochondria to maintain the membrane potential, possibly resulting in a decreased electron flux through the electron transport chain and the cytochrome oxidase. Morgan & Wikström (1991) describe the possibility of two routes of electron transfer through cytochrome oxidase, only one of which is linked to proton translocation. Should this be the case, a reduced electron flow would occur through the route linked to proton pumping. In conditions causing elevated extramitochondrial proton concentrations and reduced muscle pH therefore, this mechanism may also cause the cytochrome oxidase enzyme to receive fewer electrons and become more oxidised.

Effects of Varying O_2 Tension

Electrons carried down the ETC and reaching cytochrome oxidase are taking part in oxidative phosphorylation and aerobic energy generation. The oxygen that becomes reduced by cytochrome oxidase to form water comes from oxyhaemoglobin, and, to a much lesser extent, from O_2 dissolved in blood plasma. Changing the concentration of inspired oxygen seems to have some effect on the muscle. Hyperoxic conditions have the least effect on the various parameters measured, and are generally not significantly different from normoxic conditions. As haemoglobin is already 98%-100% saturated with O_2 at rest in normoxic conditions, it is reasonable that increasing the inspired O_2 to 100% should make little difference.

Hypoxic conditions however, had quite a marked affect on the parameters measured. As we have already seen, the heart rates were slightly higher in hypoxic conditions. Inspiring 12% O_2 will reduce the oxygen saturation of arterial blood (from 98% to approximately 70% saturation). Myoglobin has a much lower P_{50} (partial pressure of oxygen at 50% saturation) of only 5.3 mmHg, compared to 27 mmHg for haemoglobin at pH 7.4. At rest, myoglobin is approximately 70-75% saturated (Connett *et al.*, 1984; Honig *et al.*, 1992), and can fall during exercise to 20-40% saturation (Honig *et al.*, 1992; Richardson *et al.*, 1995). In describing their systems definition of aerobic capacity, Honig *et al.* (1992) discuss the low myoglobin saturation during heavy exercise being compensated for by greater redox and phosphorylation drives. A more reduced cytochrome oxidase, coupled

with a lower ATP/ADP ratio would combine to produce a greater stimulus to oxidative phosphorylation and ATP regeneration, and offset any fall in myoglobin saturation. In the present study, occluded isometric exercise seems to produce a more oxidised cytochrome oxidase. This will then leave only the lower ATP/ADP ratio to offset the fall in myoglobin saturation, and may result in a falling rate of aerobic ATP synthesis (Honig *et al.*, 1992).

During, and particularly after the cuff-occluded isometric exercise, the greatest effect on the cytochrome oxidase oxidation state was seen in hypoxic conditions. The extreme conditions of hypoxic occluded isometric exercise will cause the greatest generation of metabolites, such as lactic acid, from the muscle (Lundin & Ström, 1947). This will cause the greatest inhibition of oxidative phosphorylation and hence of the aerobic regeneration of ATP. This would be expected to lead to an even greater proportion of energy coming from anaerobic metabolism. The reduced generation of Hb in this bout of exercise (Fig. 4.6), and reduced fall in HbO_2 (Fig. 4.7), with very little change in these parameters, particularly Hb, occurring in the last 1.5 minutes of exercise (Figs. 4.8, 4.9), would all be compatible with this view. The last 1.5 minutes are then when most of the increase in oxidised cytochrome oxidase occurs (Figs. 4.11, 4.20).

Two points follow. First, this is consistent with the changes being due to occlusion causing a gradual build up of metabolites, which will show a greater effect later in exercise. Second, the fact that the majority of the increase in oxidised cytochrome oxidase occurs when very little change in Hb or HbO_2 is

taking place means that any cross-talk between the haemoglobin signal and the cytochrome oxidase signal (Cooper *et al.*, 1994) will be minimal here.

Cessation of Exercise in Occluded Muscle

Immediately after cuff-occluded exercise, with the occluding cuff still inflated, very little change is seen in Hb, or HbO_2 (Figs. 4.6, 4.7), but the cytochrome oxidase enzyme becomes even more oxidised (Fig. 4.10). Removal of the exercise stimulus to energy generation and the driving force of electrons down the ETC, will cause the cytochrome oxidase to receive fewer electrons and spend even more time waiting to be reduced. This, according to the thinking proposed above, will cause the enzyme to become significantly further oxidised. On removal of cuff-occlusion, a much greater change occurs in the Hb and HbO_2 concentrations than seen previously during contraction, presumably due to the hyperaemic effects of prior exercise. The cytochrome oxidase enzyme becomes markedly reduced as the inhibition of the aerobic energy generating pathways is removed, and electrons can flow down the ETC and reduce oxygen to water once again.

In the non-occluded exercise conditions, and the lower (30% MVC) exercise intensity with cuff-occlusion, cytochrome oxidase tended to become slightly more oxidised in the period immediately after exercise (Figs. 4.10, 4.21). In these exercise bouts, the conditions do not seem to have been severe enough to cause a significant reduction in the enzyme oxidation state due to removal of the

metabolite inhibition on cessation of exercise or on release of the occluding cuff. The effect of removal of inhibition from metabolite accumulation in the muscle, and the increase in electron supply down the ETC to cytochrome oxidase on cessation of exercise or release of the cuff, seems to have been offset by the reduction in energy demand and associated fall in electron transport required on cessation of activity, so the enzyme becomes slightly more oxidised in the period immediately after exercise.

As described earlier, some decline of handgrip force was seen in both studies. The force loss showed little relation to gas mixture breathed, to lactate produced, or to the oxidation state of cytochrome oxidase. Subjects who maintained the grip force more successfully were presumably those with a greater capacity to generate ATP anaerobically, and unrelated directly to the oxidation state of cytochrome oxidase.

The oxidation state of cytochrome oxidase also showed no correlation with lactate efflux from the muscle, estimated from venous blood lactate measured in the antecubital forearm vein. Equal amounts of lactate were produced from the two 30% exercise bouts, but only one bout caused a significant change in the cytochrome oxidase oxidation state. Blood lactate and associated metabolites will only have the inhibitory effect already described in the cuff-occluded bout of exercise, when sufficient metabolite build up will be able to occur.

Cytochrome Oxidase Concentration

Van Kuilenberg *et al.* (1991) quoted a value of 3.5 μM of cytochrome oxidase in skeletal muscle. All the values for the change of the oxidation state of cytochrome oxidase in this study are quoted as rates of change, i.e. in $\mu mol.min^{-1}$. The changes in the oxidation state of cytochrome oxidase in the recovery periods after cuff-occluded exercise, both with occlusion and after occlusion, might be taken to imply values higher than the concentration of cytochrome oxidase in human skeletal muscle. These two periods are however only 30 seconds long, so the absolute changes being described are effectively half their rate of change per minute. During hypoxic, occluded exercise, over the whole 2 minute exercise period oxidised cytochrome oxidase is seen to increase by 4.4 μM (2.2 $\mu mol.min^{-1}$ for 2 minutes, Fig. 4.10). The majority of this increase occurred in the last 1.5 minutes of the exercise period (Fig. 4.11). After an initial fall in oxidised cytochrome oxidase in the first 30 seconds, of 1.6 μM (0.8 $\mu mol.min^{-1}$ for 30 seconds), a rise of 5.4 μM was seen in the last 1.5 minutes (3.6 $\mu mol.min^{-1}$ for 1.5 minutes, Fig. 4.11). Combining the increase seen over the 2 minutes of occluded isometric contraction with the further increase in oxidised cytochrome oxidase of 3.65 μM (7.3 $\mu mol.min^{-1}$ for 30 seconds) during the 30 seconds of recovery under cuff-occlusion gives a total increase in oxidised cytochrome oxidase of 8.05 μM. This compares favourably with the decrease in oxidised cytochrome oxidase of 5.65 μM (11.3 $\mu mol.min^{-1}$ for 30 seconds) in the 30 seconds after release of occlusion, and are of the same order as the value of Van Kuilenberg *et al.*. The fact that some values are larger may of course be due to experimental error in the

technique used here. However, the possibility also exists that flexor carpi radialis contains a higher volume fraction of aerobic fibres than the muscles which were the source of the Van Kuilenberg *et al.* value (only quoted as "skeletal muscle"). Whichever the explanation, it does appear that the redox variation imposed by the manipulations described has been from almost full reduction to full oxidation.

Conclusion

This seems to finally dispel the theory of lactate production being due to a state of oxygen limitation in the muscle. The previous chapter suggested that in conditions that may promote a state of oxygen limitation, lactate efflux (and therefore presumed production) from the forearm was not more, but less, due to the muscles being able to take up the lactate, and metabolise it possibly as an energy source. This chapter seems to dissociate any link between the redox state of the muscle, and lactate production. The oxidation state of cytochrome oxidase, the most direct indicator of a state of oxygen insufficiency in the muscle, seems determined by the flux of electrons through the enzyme complex, rather than by availability of oxygen, which must be presumed to have been present in excess, even after 2 minutes of isometric exercise under occlusion.

THE OXIDATION STATE OF DYNAMICALLY EXERCISING FOREARM MUSCLE

INTRODUCTION

It is possible that oxygen never reaches limiting concentrations in muscle. The previous chapter showed that the oxidation state of muscle, as determined by the redox state of the terminal electron acceptor of the ETC, cytochrome oxidase, does vary during exercise. It seems to be determined by the flux of electrons down the ETC, and through the enzyme complex. In occluded, isometric exercise, the build up of metabolites in the muscle was thought to reduce the flow of electrons down the ETC, causing the cytochrome oxidase to become more oxidised overall.

Dynamic exercise is associated with a much higher energy demand compared to isometric exercise (Asmussen, 1981; Newham *et al.*, 1995), produced largely aerobically (Sahlin *et al.*, 1987). This will result in greater generation of reducing equivalents ($NADH_2$ and $FADH_2$), causing a greater flow of electrons down through the ETC and cytochrome oxidase. This will cause a greater amount of oxygen to be reduced to water. Will this greater oxygen utilisation in dynamic exercise cause the muscle to become oxygen limited, and the cytochrome oxidase to then become more reduced?

In the previous chapter, movement was constantly discouraged as it produced artefacts in the NIR signal. The dynamic nature of this study will obviously cause substantial movement artefact. Movement artefacts are caused by movements of the muscle under the optodes, or movement on the skin, increasing or decreasing

the light detected by the receiving optode. It was anticipated that, by rigorously controlling the movement during exercise, making it a consistent, regular contraction/relaxation pattern as defined by a metronome, the movement artefact would be regular and constant. Then by averaging 4 second periods of data from the beginning and end of the exercise bout (as used in the previous chapter, p. 129), the movement effects will cancel out. Reliable, reproducible NIRS signals will then be obtained from dynamic exercise. Movement will continue to be discouraged during the recovery periods, as in this period any movement is likely to be an isolated, irregular event.

Experiments were conducted as follows:

Subjects performed a series of three 2 minute bouts of dynamic exercise using the muscles of the forearm similar to the arm exercise used in Chapter 3. Muscle oxygenation (HbO_2, Hb, and CtOx) was monitored throughout each study via NIR spectroscopy, with a fixed recovery period between the bouts of exercise, long enough to allow the re-establishment of a steady baseline. Each subject repeated the series of exercises on a number of occasions, to monitor reproducibility.

METHODS

All experiments took place in the Exercise Physiology Research Laboratory (laboratory temperature: 20.9 ± 0.5 °C). Five subjects (4 male, 1 female) volunteered for this study (age: 31.2 ± 6.6 yr.). Each subject carried out the test on 5 separate occasions, at approximately the same time of day on each occasion.

SPECIFIC METHODS

Subjects were seated throughout the study, with one forearm supported in front of them on a flat table, and the hand protruding over the end of the table. Subjects performed the same series of simple forearm flexion exercises as used in Chapter 3 and outlined in the General Methods (Chapter 2, p. 49). The importance of regular movements during exercise was emphasised to the subject. To recap, they were instructed to move only their hand, pivoting at the wrist, lifting the weight suspended from the distal end of the handpiece. The subject kept his/her hand flat against the handpiece. Subjects were instructed to avoid using their biceps in the movement, their forearms remaining in contact with the table for this reason. This localised the exercise specifically to the forearm muscles, and minimised the movements of the forearm unconnected to the exercise. Each series of exercises involved three 2 minute bouts, at three, increasing intensities, separated by 5 minute periods of rest. The three intensities used were termed '0 kg', '1 kg', and

"'2" kg', denoting total loads of 0.1 kg, 1.1 kg, and 1.6-2.1 kg respectively. Each subject repeated the series of three forearm exercises five times.

NIRS measurements (oxyhaemoglobin, deoxyhaemoglobin, and the oxidation state of cytochrome oxidase) were recorded from the exercising forearm by optodes placed longitudinally over the flexor carpi radialis muscle on the volar surface as used previously and outlined in Chapter 2 (p. 52). Regular movement was encouraged and visually monitored during the exercise period. Unnecessary movement during both the exercise period, and the recovery period was constantly discouraged.

The subject was arranged in a comfortable position as described, and the optodes attached, before a 5 minute period of rest. Then the 2 minute periods of exercise, followed by 5 minute periods of rest began, starting at 0 kg, followed by 1 kg, and finally 1.5 or 2 kg suspended from the handpiece. The NIRS signal was recorded continuously throughout the study, at 1 sample per second.

RESULTS

As mentioned in the Introduction, the NIRS data were analysed as previously described by taking the difference between a 4 second period of averaged data at the beginning of exercise, and a 4 second period at the end of exercise, taking the difference and calculating a rate of change (Fig. 5.1).

Statistical Analysis

The effect of the increasing exercise intensity was assessed using a two-way analysis of variance technique. Significant differences were then determined by Bonferroni follow-up multiple comparison tests. Statistical significance was taken at the 5% level, and illustrated graphically by an asterisk (*). The reproducibility of the data was analysed using confidence intervals.

Deoxyhaemoglobin (Hb), Oxyhaemoglobin (HbO_2)

The rate of Hb increase became significantly greater as exercise intensity increased, in a regular manner to a maximum rate of 16.0 ± 1.2 $\mu mol.min^{-1}$ during the most intensive bout (Fig. 5.2). The large majority of the increase occurred in the first 30 seconds, with the rate of Hb accumulation again increasing

significantly with exercise intensity (Fig. 5.3). No significant rate of change occurred in the last 1.5 minutes of each 2 minute bout. In the first minute after each exercise, the rate of Hb production fell (Fig. 5.4). It fell significantly faster with each bout of increasing exercise intensity, reaching a maximum rate of 4.7 ± 0.4 $\mu mol.min^{-1}$ after the "2" kg bout.

HbO_2 fell quicker with increasing exercise intensity. The rate of decrease only became significant however, in the final "2" kg bout, reaching 5.9 ± 1.0 $\mu mol.min^{-1}$ (Fig. 5.5). The rate of HbO_2 decrease during the intermediate intensity bout of exercise at 1 kg (1.1 ± 0.7 $\mu mol.min^{-1}$), was less than the slight rise in the easy, initial 0 kg bout (0.4 ± 0.4 $\mu mol.min^{-1}$), but not significantly so. Again, the majority of the change occurred in the first 30 seconds (Fig. 5.6). The final 1.5 minutes of each 2 minute bout showed little change. The HbO_2 increased significantly faster in the final 1.5 minutes of the "2" kg bout of exercise than in the either of the two easier bouts. During the first minute of recovery after each bout of exercise, the HbO_2 fell significantly, but showed no significant effect due to exercise intensity. After the final, maximal bout of exercise at "2" kg however (Fig. 5.7), the HbO_2 showed the slowest decline (0.5 ± 0.1 $\mu mol.min^{-1}$), but was marginally not significantly smaller than the earlier, easier bouts.

Cytochrome Oxidase (CtOx)

The oxidation state of the cytochrome oxidase enzyme showed no significant change during easy 0 kg exercise (Fig. 5.8). It became significantly reduced however, during the two more intensive bouts (at rates of 0.5 ± 0.1 $\mu mol.min^{-1}$ and 0.5 ± 0.1 $\mu mol.min^{-1}$ during the 1 kg and "2" kg bouts respectively). However, the two more intensive bouts were not significantly different. Similar to the changes seen in the Hb and HbO_2 data, the majority of the change occurred in the first 30 seconds of exercise. Only the 1 kg bout achieved any significance, the cytochrome oxidase enzyme becoming statistically more rapidly reduced than during the 0 kg bout (Fig. 5.9). Little change occurred in the final 1.5 minutes, but in the most intensive "2" kg bout, the cytochrome oxidase became significantly more rapidly reduced compared to the last 1.5 minutes of the easy 0 kg intensity bout (Fig. 5.9). Little change was seen in the first minute of recovery (Fig. 5.10), and there was no significant effect of exercise intensity.

Reproducibility

The nature of this study allowed the within-subject variation to be calculated. The reproducibility of the Hb, HbO_2, and CtOx NIRS data can be shown by using the 95% confidence intervals for each variable at each intensity (Fig. 5.11). At the easiest workload, the NIRS variables show a small rate of change. As the work load increases, the physiological demand on the exercising muscle increases, and

the mean value increases or decreases from zero. The variation and reproducibility of the data is illustrated by 95% confidence intervals around these mean values. These remain similar over all the exercise intensities, and also between the different NIRS variables, relative the the mean value.

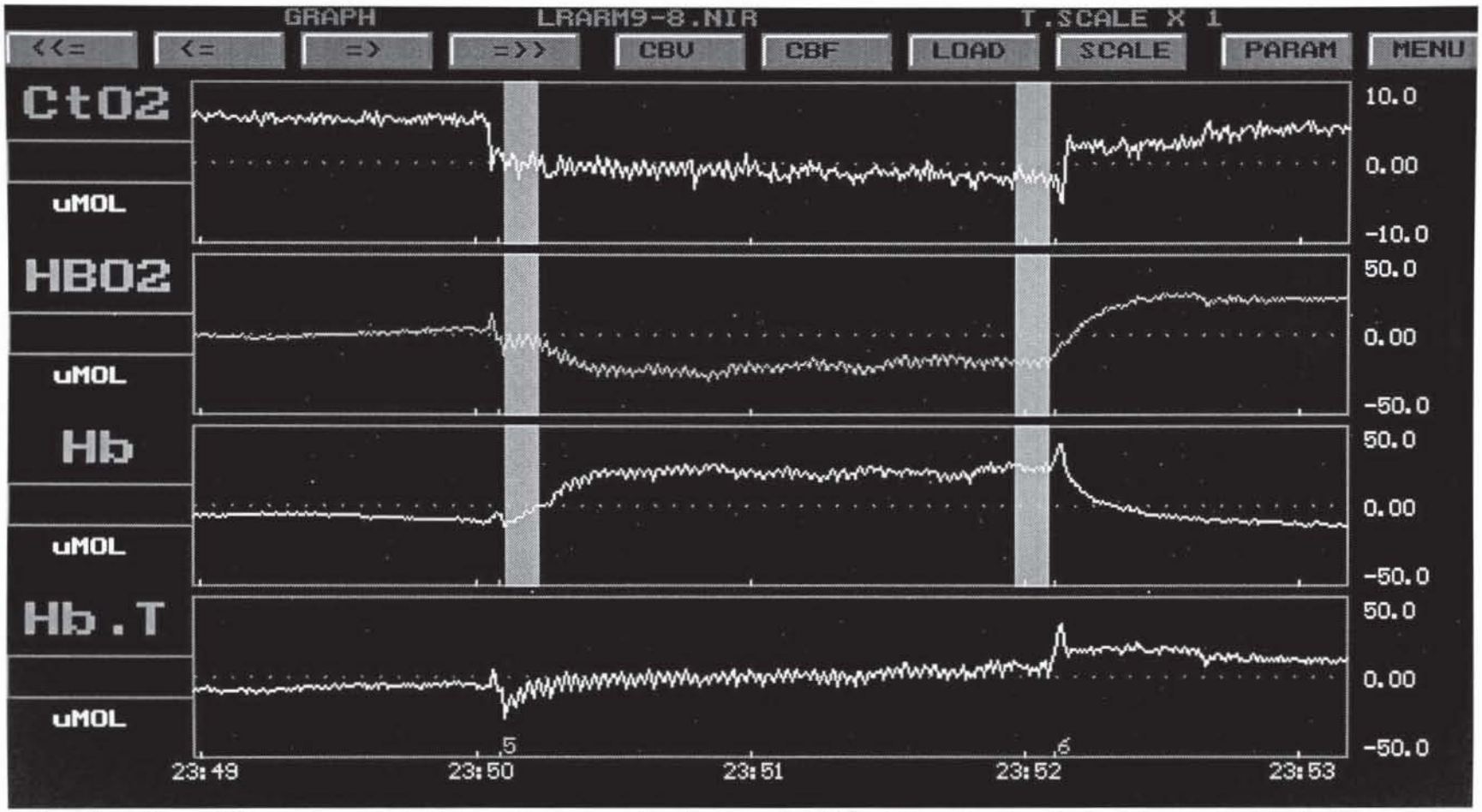


Fig. 5.1: Trace of 2 minute period of dynamic forearm flexion exercise lifting 2 kg, showing the two 4 second periods of analysis at the start and end of exercise. Headings on the left refer to oxidised cytochrome oxidase (CtO2), Oxyhaemoglobin (HbO2), Deoxyhaemoglobin (Hb), and Total Haemoglobin (Hb.T [= HbO2 + Hb]). Values on the right are full scale deflection concentration changes in mM, from zero at rest.

Fig. 5.2

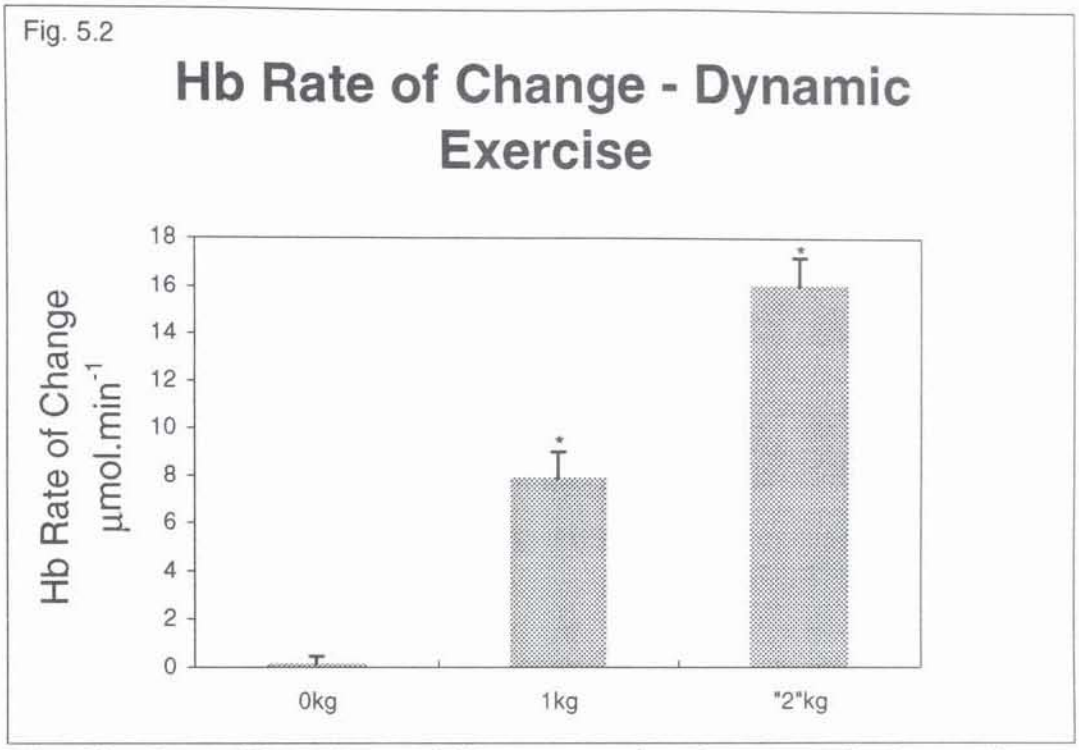


Fig. 5.2: Rate of Deoxyhaemoglobin concentration change over 2 minutes of dynamic forearm exercise at 3 different intensities.

Fig. 5.3

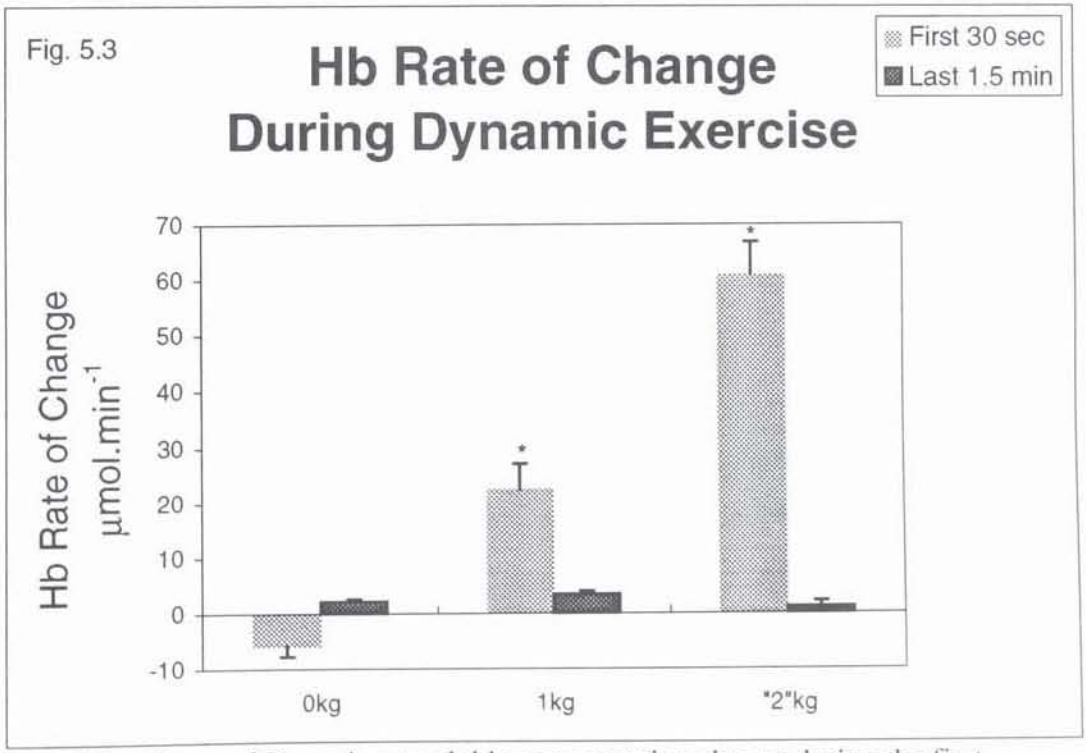


Fig. 5.3: Rates of Deoxyhaemoglobin concentration change during the first 30 seconds and last 1.5 minutes of the 2 minute period of dynamic forearm exercise at 3 differerent intensities.

Fig. 5.4

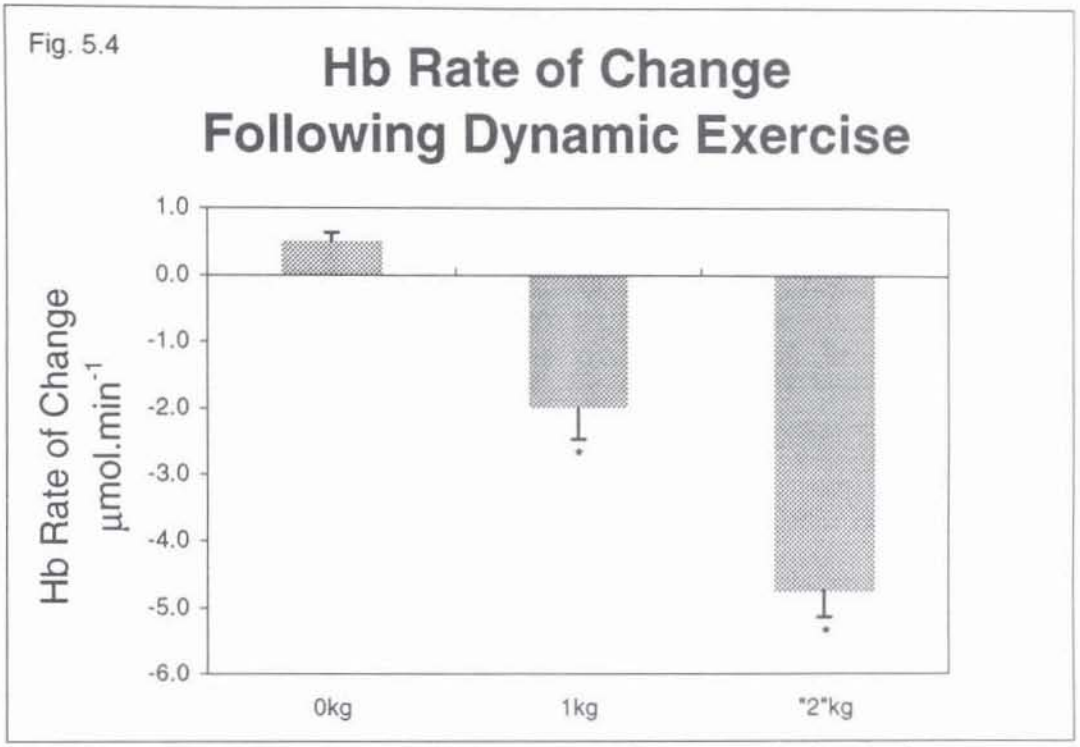


Fig. 5.4: Rate of Deoxyhaemoglobin concentration change in the 1 minute following dynamic forearm exercise at 3 different intensities.

Fig. 5.5

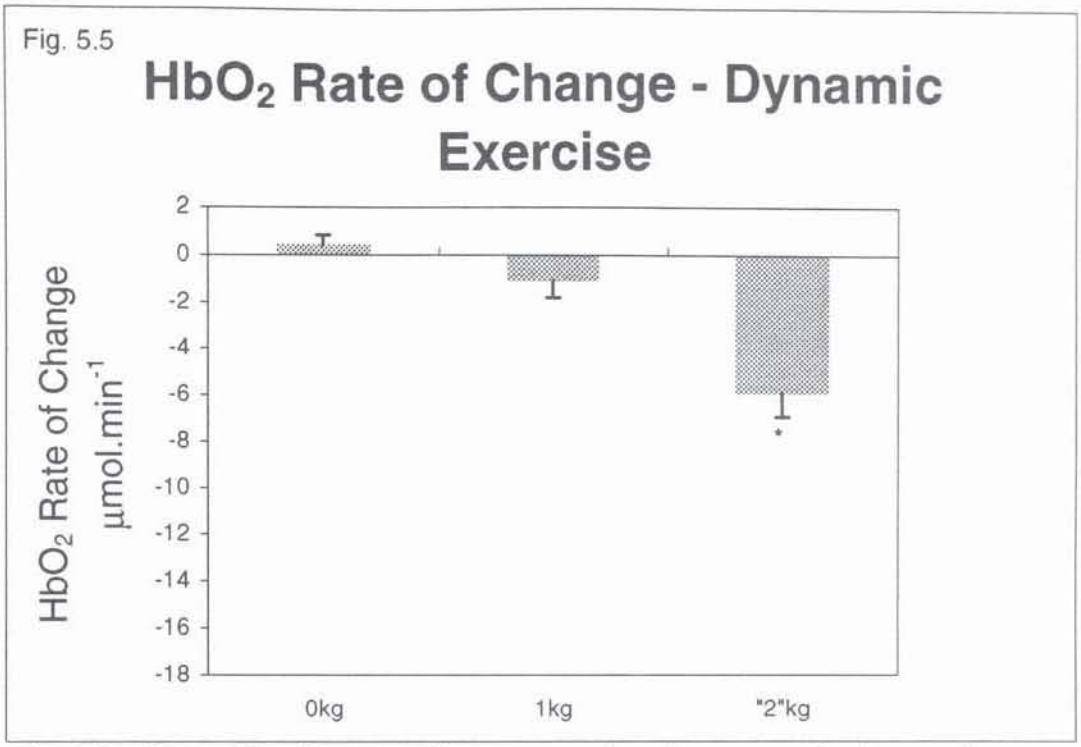


Fig. 5.5: Rate of Oxyhaemoglobin concentration change over 2 minutes of dynamic forearm exercise at 3 different intensities.

Fig. 5.6

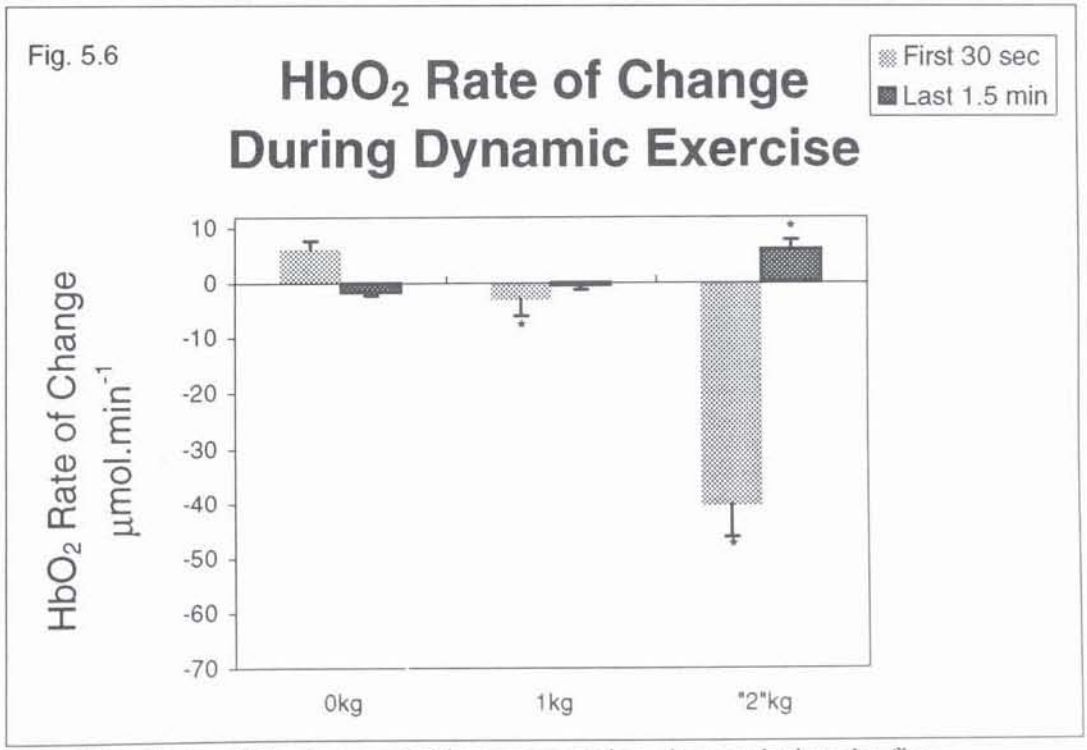


Fig. 5.6: Rate of Oxyhaemoglobin concentration change during the first 30 seconds and last 1.5 minutes of the 2 minute period of dynamic forearm exercise at 3 different intensities.

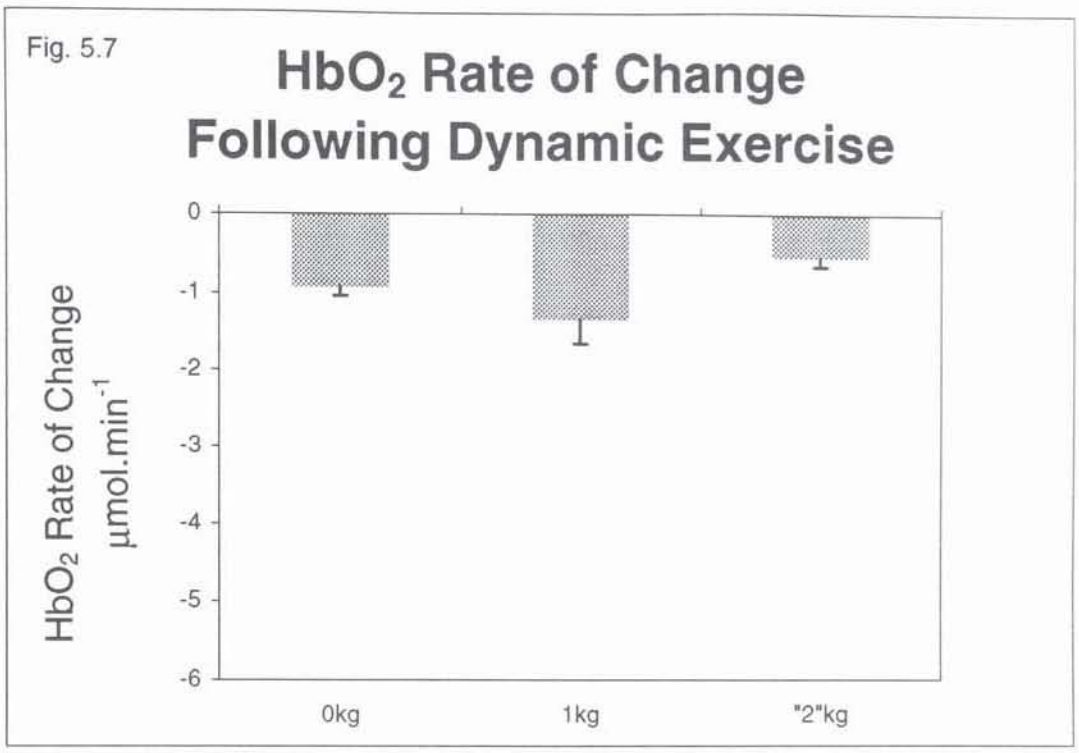


Fig. 5.7: Rate of Oxyhaemoglobin concentration change in the 1 minute following dynamic forearm exercise at 3 different intensities.

Fig. 5.8

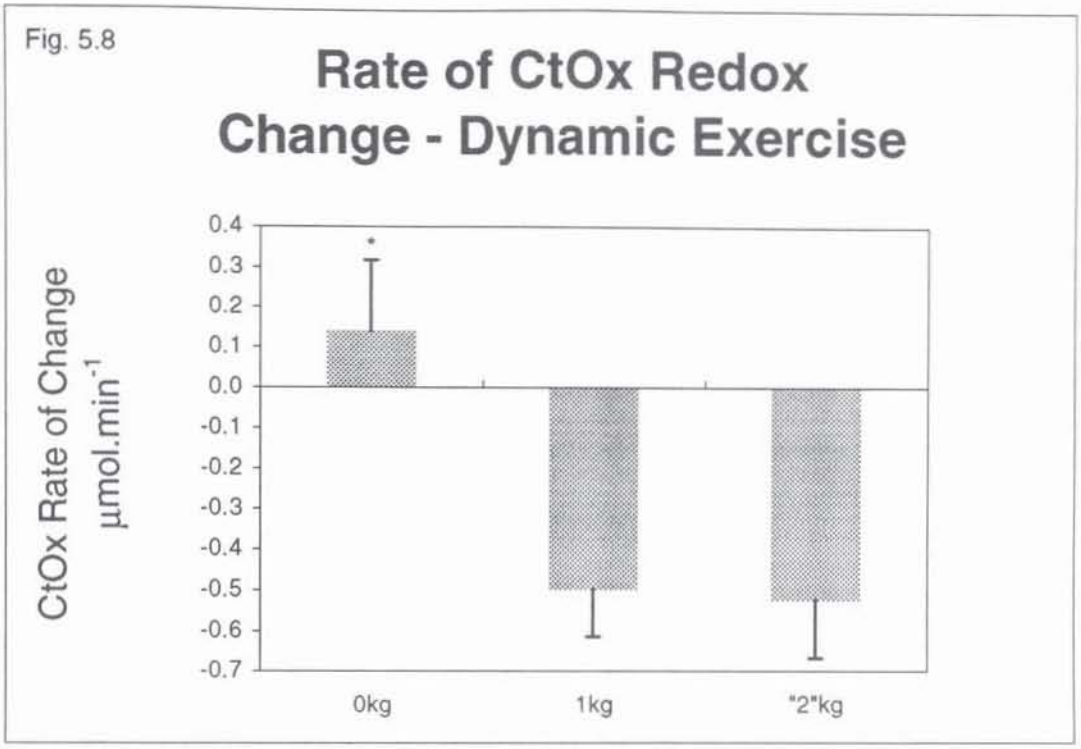


Fig. 5.8: Rate of Cytochrome Oxidase oxidation state change over 2 minutes of dynamic forearm exercise at 3 different intensities.

Fig. 5.9

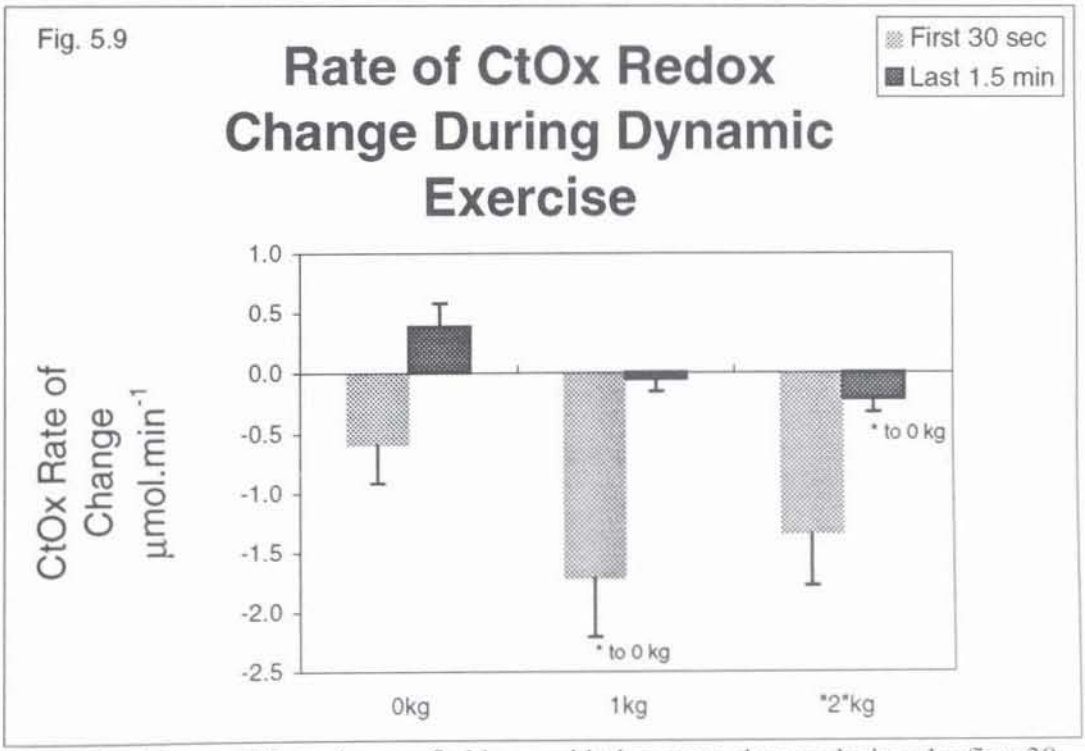


Fig. 5.9: Rates of Cytochrome Oxidase oxidation state change during the first 30 seconds and last 1.5 minutes of the 2 minute period of dynamic exercise at 3 different intensities.

Fig. 5.10

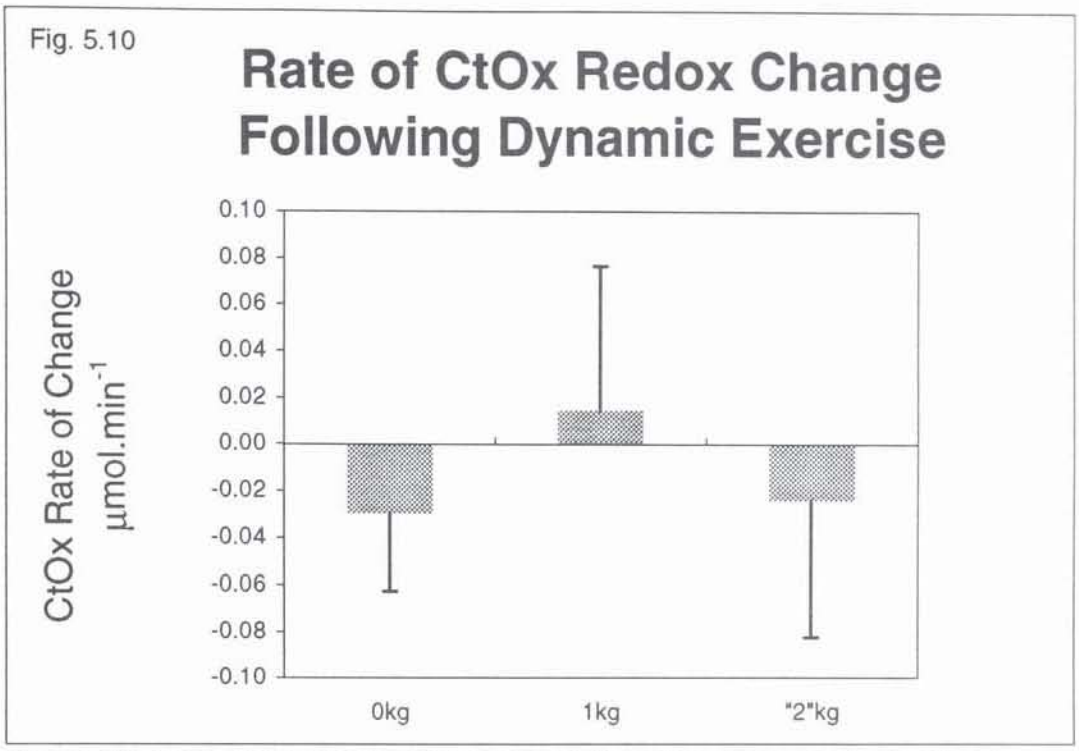
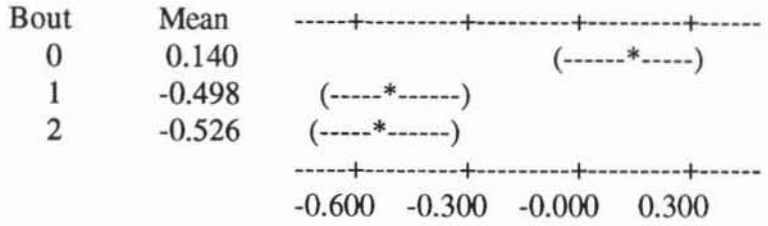


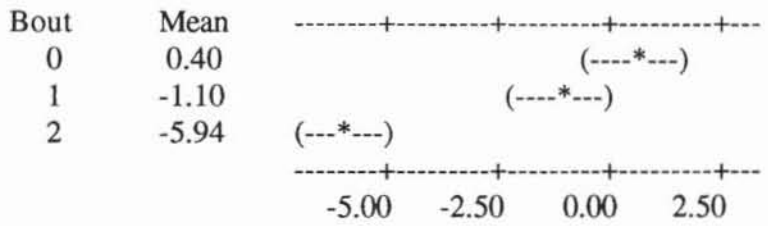
Fig. 5.10: Rate of Cytochrome Oxidase oxidation state change in the 1 minute following dynamic forearm exercise at 3 different intensities.

Fig. 5.11: Individual 95% Confidence Intervals for the Rates of Change in the NIRS Variables at each Work Intensity.

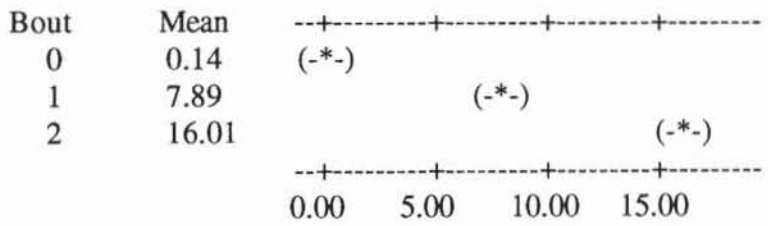
Cytochrome Oxidase Rate of Change, $\mu mol.min^{-1}$:



Oxyhaemoglobin Rate of Change, $\mu mol.min^{-1}$:



Deoxyhaemoglobin Rate of Change, $\mu mol.min^{-1}$:



DISCUSSION

Due to the susceptibility of the near infrared signal to movement, studies of dynamically exercising muscles had previously been difficult. By adopting a regular, defined movement pattern, and using the averages of 4 second periods of data, it was hoped that representative NIRS values could be obtained for changes in oxyhaemoglobin, deoxyhaemoglobin, and the oxidation state of cytochrome oxidase during dynamic exercise. By repeating a series of exercises performed by a group of 5 subjects, 5 times each, the reproducibility and reliability of the NIRS data could be assessed.

Reproducibility

The 95% Confidence Intervals for the data groups gave a reliable indication of the within subject variation, independent of the size of the mean value (Fig. 5.11). The Coefficient of Variation, a statistic often used to show repeatability and precision in measurements, has been criticised as its value depends on both the standard deviation and the mean - small means producing large coefficients of variation (Bland, 1987). The standard deviation for the data sets was reasonably constant, but the mean values varied widely. At the easiest exercise intensity there was very little physiological demand, and the resulting change on the NIRS variables was very close to zero. As the workload increased, a larger physiological response was detected by NIRS. This would produce large variations if the

reproducibility were expressed as coefficients of variation, due to the changing mean values.

The 95% confidence intervals either side of the mean values for the data groups show clearly the reproducibility for each workload (Fig. 5.11). The signals in all three variables studied (Hb, HbO_2, and CtOx) seem to have equal variability relative to the mean in each of the three exercise periods. The changes in Hb and HbO_2 have been accepted as valid, reliable indicators of physiological changes (Mancini *et al.*, 1994). The same order of variability applied to the much lower cytochrome oxidase signal may render it unreliable in clinical applications, where once-off readings have to be trustworthy. However, by analysing a group of subjects under the same conditions, it seems reasonable to conclude that the mean result will give as accurate and reproducible an indication of the changes in cytochrome oxidase oxidation state as those obtained for Hb and HbO_2. The average standard error values from the 2 minute bouts of dynamic exercise (2.6 for Hb, 2.1 for HbO_2, and 0.4 for the CtOx changes) agree well with the average values from the previous chapter involving isometric exercise (2.2 for Hb, 2.7 for HbO_2, and 0.5 for the CtOx changes). In conclusion, by comparing averages of 4 second periods of data from the start and end of the 2 minute exercise bout, accurate indications of the physiological changes seen in Hb, HbO_2, and CtOx during both isometric, and dynamic exercise can be obtained.

Deoxyhaemoglobin, Oxyhaemoglobin

The majority of energy in dynamic exercise is generated by aerobic metabolism (Sahlin *et al.*, 1987). The oxygen used in this process is carried to the exercising muscle by haemoglobin in the red blood cells. As the exercise intensity increases, the energy demand increases, so the oxygen utilisation from oxyhaemoglobin (HbO_2) increases, causing the production of more deoxyhaemoglobin (Hb). The rate of production of Hb increased in approximately equal steps through easy, moderate, and hard exercise (Fig. 5.2). As Hb can only be formed from HbO_2 when the oxygen is used, Hb production can be taken as an indication of oxygen utilisation by the muscle. From the Hb production in each exercise bout, the three exercise intensities seemed to accurately fit the study objectives of three equally spaced exercise intensities - an easy exercise, a hard exercise, and one midway between.

As the exercise intensity increases, the muscle will adapt to the increased energy demand by increasing its blood supply. This explains the failure to get a regular decrease in HbO_2 in the muscle as exercise intensity increases. Similar to the reasons for the unequal rise in Hb and fall in HbO_2 outlined in the previous chapter, as exercise intensity and oxygen demand increase, so will HbO_2 supply. The very small rate of fall in HbO_2 in the intermediate 1 kg exercise bout (Fig. 5.5) suggests the muscle vasculature can quickly adapt to that intensity. Thus, the increase in oxygen utilisation (deoxygenating HbO_2) was offset by an equally rapid increase in oxygen supply (fresh HbO_2). Only in the most intensive bout,

which was the maximum subjects could manage for 2 minutes, is there a significant fall in HbO_2. This suggests the vasodilation mechanisms of the muscle cannot adapt as quickly or completely as in the easier intensities, and greater oxygen extraction per unit of blood must therefore result.

That the muscle adapts quickly to the exercise intensities imposed on it is shown in Figs. 5.3 and 5.6. A steady state of Hb production, and therefore oxygen utilisation is achieved in the first 30 seconds of each bout of exercise, and very little change occurs in the last 1.5 minutes (Fig. 5.3). The majority of change in the HbO_2 also occurs quickly, suggesting the muscle vasculature has adapted to the increase in muscle oxygen demand within 30 seconds of starting exercise (Fig. 5.6). There is even a small increase in HbO_2 in the last 1.5 minutes of the most intensive stage. This could be due to continuing vasodilation beyond the first 30 seconds, increasing the HbO_2 delivered to the muscle. The large fall in HbO_2 during the initial 30 seconds of the most intensive bout can be attributed to an increased oxygen extraction before the vasculature has adapted.

On completion of the 2 minute exercise periods, both the Hb and HbO_2 concentrations fell (Figs. 5.4, 5.7). The Hb falls in a regular manner, similar to its rise during exercise, with the most rapid fall in the first minute post exercise occurring after the most intensive bout. On cessation of activity a reduction in oxygen/energy demand will result, causing a fall in Hb generation, which will be greatest after the most intensive exercise bout. This fall is numerically not as large as the rises seen during the respective exercise bouts, indicating incomplete return

to resting levels of metabolism and hence oxygen uptake by the end of 1 minute post exercise.

The HbO_2 also falls (Fig. 5.7), suggesting the reduction in blood flow in the post exercise period is sufficient to offset the decrease in oxygen demand and therefore increase in HbO_2 delivery. There was no statistically significant difference between the effects of different exercise bouts. In the minute following the most intensive bout of exercise, the fall in HbO_2 was at a slightly lower rate than after both the easy 0 kg bout, and the intermediate 1 kg bout. This is consistent with the more intensive exercise causing a more prolonged maintenance of exercise hyperaemia in recovery, keeping the forearm blood flow and HbO_2 supply high.

Cytochrome Oxidase Oxidation State

During exercise, the cytochrome oxidase becomes more reduced with increasing dynamic exercise intensity (Fig. 5.8). The opposite changes in oxidised cytochrome oxidase reported in this chapter compared to the previous one (Figs. 4.10, 4.19, 5.8) occur despite similar changes in Hb and HbO_2 (Figs. 4.6, 4.7, 4.13, 4.16, 5.2, 5.5). This makes the possibility of cross-talk from the haemoglobin signals, and of random scatter, unlikely to be responsible for the changes seen in the CtOx signal. It suggests they reflect true physiological changes occurring in the muscle. This is consistent with the hypothesis of cytochrome oxidase oxidation state being determined by flux of electrons through

the enzyme complex. It is also consistent with a situation of oxygen-limitation. This latter point is discussed later.

Dynamic exercise, although obtaining the majority of its energy from aerobic metabolism (Sahlin *et al.*, 1987), requires much more energy than isometric exercise (Asmussen, 1981; Newham *et al.*, 1995). The maximum blood lactate concentration from the exercising arm after the most intensive "2" kg bout of arm-only exercise in Chapter 3 was 2.36 $mmol.l^{-1}$. The maximum concentration obtained from isometric exercise in the last Chapter was 1.78 $mmol.l^{-1}$. More lactate was produced in the dynamic exercise even though it only accounts for 2% of the total energy generated (Sahlin *et al.*, 1987). However, free blood flow access to the exercising forearm was maintained throughout dynamic exercise, so the effects of metabolite build-up seen in the occluded bouts of isometric exercise could not occur.

As the exercise intensity rises, greater energy demand will cause a greater flow of electrons down the ETC, and through the cytochrome oxidase complex. Little change is seen in the easiest intensity 0 kg bout, but during the moderate and hard exercise bouts, the cytochrome oxidase redox state becomes significantly reduced, falling to similar levels in the two instances. Again, the majority of this change occurs in the first 30 seconds of exercise (Fig. 5.9), consistent with a steady state level of energy production/utilisation being reached. During both moderate and hard exercise bouts, the level of oxidised cytochrome oxidase fell by approximately 1.0 μM (average rate of fall of 0.5 $\mu mol.min^{-1}$ for 2 minutes) over

the 2 minute bout. The fact that moderate intensity exercise, and hard intensity exercise both reduced the cytochrome oxidase by the same amount, largely within the first 30 seconds of the 2 minute bouts, suggests the enzyme may be 100% reduced in both cases. This fall can be compared with a maximum increase in oxidised cytochrome oxidase of 8.05 μM over the hypoxic, occluded isometric exercise bout in the previous chapter (Fig. 4.10). Cytochrome oxidase was assumed to be near 100% oxidised at this point. The start of both periods of analysis was rest, or not significantly different to rest (from the point of view of the oxidation state of cytochrome oxidase). The smaller fall in dynamic exercise compared to the greater rise in occluded, isometric exercise is consistent with the enzyme complex being in a more reduced state at rest.

The majority of change occurring in the first 30 seconds of exercise is consistent with the changes being linked to increases in energy demand. In the previous chapter, a much larger change was seen in the latter part of exercise, particularly in the occluded, hypoxic condition (Figs. 4.11, 4.25). This is consistent with the change being due to an accumulated effect such as the progressive build-up of metabolites during exercise. This will cause a greater effect with increasing exercise duration.

As noted several times previously, Van Kuilenberg *et al.* (1991) quoted values of 3.5 μM of cytochrome oxidase in muscle. From rest to fully reduced, the level of oxidised cytochrome oxidase falls by approximately 1.0 μM (0.5 $\mu mol.min^{-1}$ for 2

minutes). Assuming Van Kuilenberg *et al.*'s estimation of cytochrome oxidase content of muscle is correct at 3.5 μM, this puts the resting enzyme in a state of:

$$\frac{1.0\,\mu M}{3.5\,\mu M} \times 100 = 29\,\%\ \text{oxidised}$$

This seems acceptably similar to the values of Hempel *et al.* (1977) quoted in the previous chapter, of 85% reduction, but which was carried out on resting cat brain. It also agrees closely with the theoretical value calculated in the previous chapter, of cytochrome oxidase in muscle being 50-75% reduced at rest.

From the results of the previous chapter, the cytochrome oxidase enzyme became a total of approximately 8.05 μM more oxidised. When combined with the 1 μmol fall in oxidised cytochrome oxidase seen from resting in the present study, this makes a possible total concentration of cytochrome oxidase in the muscle of 9.05 μM. These calculations suggest that Van Kuilenberg may have underestimated the cytochrome oxidase content of muscle. However, in their study comparing the performance of several NIRS algorithms, Matcher *et al.* (1995) commented that the changes in the cytochrome oxidase redox state calculated using the algorithm used in the Hamamatsu NIRO-500 may slightly overestimate the changes detected by a continuous wavelength charge-coupled device (CCD)-based tissue spectrophotometer, assumed to accurately detect the real physiological changes. Nevertheless, all calculations in this thesis must necessarily be based on the values given by the NIRO-500.

If the enzyme can become 1.0 μM further reduced from its resting state, and using the value of approximately 9.0 μM as the total cytochrome oxidase content of muscle, then at rest, 8.0 μM of the cytochrome oxidase population is reduced, giving 89% reduction.

If cytochrome oxidase is completely reduced during moderate to hard exercise, the possibility arises of the extra energy demand being met from anaerobic sources. However this is counter-indicated by the continued rise in Hb generation in the hard exercise bout (Fig. 5.2), which shows the oxygen utilisation and aerobic energy generation are continuing. This suggests that the muscle is not in a state of oxygen-limitation, so the possibility that the changes seen in the cytochrome oxidase oxidation state are due to oxygen lack is rejected. Nevertheless, cytochrome oxidase has been shown to display first order kinetics with respect to cytochrome c concentration, or electron supply, throughout the physiological range of conditions (Smith & Conrad, 1956; Minnaert, 1961). Thus, an increase in aerobic energy generation with an unchanging redox state of cytochrome oxidase at first seems paradoxical.

The respiratory enzymes, and cytochrome oxidase in particular, are complex enzymes whose properties have been the subjects of much work and discussion. There now seems to be a consensus of opinion on the route of electrons through cytochrome oxidase (Denis, 1986; Hill *et al.*, 1986; Cooper, 1990; Malmström, 1990; Cope, 1991; Babcock & Wikström, 1992; Cooper *et al.*, 1994). As noted in the previous chapter, electrons from cytochrome c are passed to the cytochrome a

(haem a)/Cu_A site (the electron acceptor site), then on to the cytochrome a_3 (haem a_3)/Cu_B site (the oxygen binding site). The details of the enzyme mechanism however, such as the kinetics of electron transfer and the proton pumping function remain topics of current debate. Several models have been suggested to account for the enzyme mechanism. The model proposed by Bickar *et al.* (1986), can accurately explain many of the changes seen in cytochrome oxidase enzyme kinetics, and can explain the aerobic energy generation paradox seen here. It assumes a relatively rapid two-electron transfer between the cyt a/Cu_A and the cyt a_3/Cu_B sites, and a thermodynamic equilibrium in the resting enzyme that favours reduced cyt a/Cu_A, and oxidised cyt a_3/Cu_B. The two electron accepting sites of cytochrome oxidase therefore have different electron affinities. Near Infrared Spectroscopy detects the redox state of cytochrome oxidase from the copper atom at the electron acceptor site (Cu_A - Cope, 1991; Cooper *et al.*, 1994). This site will be largely reduced at rest, with an electron, and may only be capable of becoming slightly further reduced as the electron flux increases during exercise. The first order kinetics of cytochrome oxidase throughout the physiological range of electron supply (Smith & Conrad, 1956: Minnaert, 1961) suggests that as the electron flux and oxygen demand increases, the oxygen binding site of cytochrome oxidase may display a gradually increasing level of reduction. As more electrons reach the oxygen binding site, more oxygen per unit time will be reduced to water, but oxygen itself may never reach limiting levels.

The Bickar *et al.* (1986) model accounts for the cytochrome oxidase moiety being studied by NIRS being largely reduced at rest; consequently, at moderately

intensive exercise and above, it can only become slightly further reduced. Cytochrome oxidase requires both cytochrome c as an electron donor, and O_2 as an electron acceptor to achieve its maximum rate of internal electron transfer. As the exercise intensity, and the aerobic energy demand increases, the generation of reducing equivalents $NADH_2$ and $FADH_2$ will increase, raising the electron pressure onto cytochrome oxidase from cytochrome c. Cytochrome c seems specific to cytochrome oxidase, as other reductants slow electron transfer in cytochrome oxidase by up to 100 times (Bickar *et al.*, 1986). Oxygen is capable of rapid binding to the oxygen binding site, and causes a large increase in the rate of electron transfer through the enzyme. In conditions of very light exercise, energy demand will be very low. Conditions will not be appreciably different from rest, when the thermodynamic equilibrium of the enzyme favours a more reduced electron accepting site (cyt a/Cu_A site), so no significant redox state change from rest will be seen. In conditions of moderate exercise intensity and above, the increased electron pressure onto cytochrome oxidase from cytochrome c, and the increased blood flow supplying O_2 to the exercising muscle, will combine to increase the rate of electron transfer through cytochrome oxidase (raising the 'redox drive' of Honig *et al.*'s Systems View of Aerobic Capacity, 1992). This will allow the increase in the rate of aerobic energy generation to occur. As the electron accepting site of the enzyme is largely reduced at rest however, this will result in only a small further reduction in the cytochrome oxidase redox state as determined by NIRS, and with no further significant change between moderate and hard intensities.

BLOOD FLOW, AND THE OXIDATION STATE OF HAEMOGLOBIN AND CYTOCHROME OXIDASE IN A SMALL MUSCLE GROUP EXERCISING BOTH ALONE, AND SIMULTANEOUSLY WITH A LARGE MUSCLE GROUP

INTRODUCTION

Exercising muscles produce lactate at exercise intensities over approximately 50% $\dot{V}O_{2max}$ (Knuttgen & Saltin, 1972; Bang, 1936). If presented with arterial blood containing a sufficiently high concentration of lactic acid, the exercising muscle is capable of taking up the lactic acid, despite possible reductions in blood flow and oxygen supply to the working muscle (Chapter 3).

The muscle itself is unlikely to ever become oxygen limited (Jöbsis & Stainsby, 1968; Connett *et al.*, 1984, 1986; Stainsby *et al.*, 1989). The redox state of the muscle does change however, depending on the conditions of the exercising muscle, and the mode of exercise. In isometric forearm exercise with an occluding cuff around the upper arm, the exercising muscle effectively becomes a closed system. Metabolites from the exercising muscle will build up, causing the flux of electrons through the Electron Transport Chain (ETC) and through the cytochrome oxidase enzyme complex to gradually fall. This will cause the terminal cytochrome to become paradoxically more oxidised (Chapter 4). In dynamic exercise, the greater energy demand causes an increased generation of reducing equivalents, and an increased flow of electrons down through the ETC and through the terminal enzyme complex - a process which can continue more freely because maintained blood perfusion counteracts any build-up of inhibitory products. This causes the cytochrome oxidase enzyme to become more reduced (Chapter 5). How will the redox state of muscle change in dynamic exercise, in conditions of lactate uptake by the exercising muscle?

The protocol of Chapter 3 involving a series of forearm flexion exercise bouts superimposed upon increasing leg cycling intensities, caused the lactate concentration in the blood supplying the exercising forearm muscle to gradually rise. At the most intense exercise combination used, when the arterial lactate was highest, the forearm muscle even though exercising was in a state of zero lactate production, or net lactate uptake.

In the study described in this chapter a similar protocol was used as that adopted in Chapter 3. The energy demand for the forearm exercise intensities will not change when repeated during increasing leg exercise intensity. Lactate that is taken up by exercising muscle is thought to be utilised, perhaps at the expense of glucose, to generate energy (Richter *et al.*, 1988). This being the case, the cytochrome oxidase redox state will not directly change. It will simply become equally reduced at the heavier arm exercise intensities, regardless of the leg exercise intensity.

However, the increasing cytoplasmic lactate may affect the muscle mitochondrial environment. This may cause a gradually decreasing level of cytochrome oxidase reduction, reducing the flux of electrons necessary or possible down the ETC. The exercising muscle's cytochrome oxidase would then become less reduced as leg exercise intensity and the arterial blood lactate concentration increased.

Blood flow was also monitored in this study. The original assumptions upon which the rational of both this study and Chapter 3 were based, that blood flow to a small exercising muscle mass (the forearm) would be challenged by the competing demands of a large muscle mass (the legs) exercising at increasing intensities, was evident only to a small degree in the original study. One of the reasons for this was thought to be the fact that the earliest possible blood flow measurement was approximately 15 seconds after the end of exercise. Post-exercise hyperaemia may have had a complicating effect here. In the study now reported, the protocol was redesigned to allow a period in which the legs were still active, but the forearm had ceased exercise. In this period, an additional blood flow measurement was taken. It was hypothesised that this would show a clear reduction in blood flow as the leg exercise intensity increased and "stealing" of blood flow from the actively exercising forearm occurred.

Experiments were performed as follows:

Subjects carried out a series of forearm flexion exercises at increasing intensities. They then superimposed these exercises onto the legs also exercising at increasing intensities. The effect of this on the blood flow and the NIRS signals was assessed.

METHODS

All experiments took place in the Exercise Physiology Research Laboratory (laboratory temperature: 20.9 ± 0.5 °C; barometric pressure: 765.6 ± 1.6 mmHg). Ten subjects (9 male, 1 female) who were at least recreationally fit, and partaking in regular exercise, volunteered for this study (age: 25.4 ± 1.7 years; weight: 67.2 ± 2.2 kg; height: 176.9 ± 1.9 cm; aerobic capacity ($\dot{V}O_{2max}$): 59.3 ± 2.5 $ml.kg^{-1}.min^{-1}$).

SPECIFIC METHODS

This study was essentially a repeat of the study used in Chapter 3, superimposing forearm exercise onto supine cycling. In this study however, the near infrared signals from the exercising arm (oxyhaemoglobin, deoxyhaemoglobin, cytochrome oxidase redox state) were monitored instead of antecubital forearm vein and earlobe 'arterial' blood lactate concentrations.

Subjects were positioned lying supine throughout the study. Supine cycling was carried out on a Monark 818E mechanically braked cycle ergometer adapted for supine cycling. The supine ergometer closely fitted the dimensions of the Siemens Ergomed 740L Couch Ergometer used previously (Siemans Ergometer - crank length: 17 cm, axle height: 33 cm, axle width: 15 cm; Monark Supine Ergometer - crank length: 17 cm, axle height: 36.5 cm, axle width: 12 cm; ball of foot

positioned over axle of pedal in both cases).' The only substantive difference between the two systems was that the Monark adapted supine cycle ergometer was not pedal-cadence independent.

As in chapter 3, the protocol consisted of two parts. The first part is a discontinuous maximum oxygen consumption/power output test, and is exactly the same as that used in Chapter 3. It is described in detail in the General Methods (Chapter 2, p. 46). The second part involved the same basic principle as used in Chapter 3, of a dynamically exercising forearm performing simple wrist flexion either alone or superimposed upon both legs cycling, all in the supine position (described in Chapter 2, p. 48).

Heart rate was again recorded throughout. The NIRS measurements were recorded from the exercising forearm by optodes attached to the volar surface of the forearm, over the flexor carpi radialis muscle as before (Chapter 2, p. 52). The NIRS data was recorded at 2 samples per second throughout the study, and stored on an IBM compatible 486-25 MHz computer.

The subjects located themselves on the supine cycle and were attached to the NIR spectrophotometer before a 5 minute period of complete supine rest. A blood flow measurement was then taken before a 5 minute leg warm-up period at 60W. Blood flow was recorded only in the exercising arm. It was calculated from the initial rate of increase of the total haemoglobin NIRS signal (oxyhaemoglobin + deoxyhaemoglobin) under venous occlusion, based on the method of De Blasi *et*

al. (1994), and as outlined in the General Methods, Chapter 2 (p. 54). Venous occlusion was achieved using a cuff around the upper part of the exercising arm inflated to 50 mmHg, as used in venous occlusion strain gauge plethysmography. Venous occlusion pressure was achieved in under 0.3 second, and held for 10-15 seconds.

Following the leg warm-up was another 5 minute period of rest, in which blood flow was measured on two further occasions, at 15 seconds from the end of exercise and again after 2 minutes. The sequence of 2 minute periods of arm-only exercise separated by 5 minute periods of rest then began. The forearm movement was the same as used previously (Chapter 3 and 5, and described in Chapter 2, p. 49), and was again rigorously controlled as described in Chapter 5. The forearm was supported at body height by a cradle positioned to the side of the supine cycle.

Blood flow was measured twice after each bout of arm-only exercise, after 15 seconds of rest then again after 2 minutes. The forearm exercises were then repeated, superimposed on top of 4 minute periods of supine cycling. As in Chapter 3, the supine cycling intensities were 30%, 60%, 90%, then 30% again of the subjects' own individual maximum, calculated previously.

At this point, the actual protocol was modified slightly in the present study from that used in Chapter 3. The 4 minute bouts of exercise involved leg-only exercise for 1.75 minutes, then leg+arm exercise for the next 2 minutes. In the final 15

seconds of the 4 minute exercise period, the arm stopped exercising but the legs continued to cycle. During this period, an additional arm blood flow measurement was made. After 4 minutes the exercise ceased, and 5 minutes of rest ensued, blood flow being measured after 15 seconds and again after 2 minutes as previously described.

The measurements during recovery were designed to copy the original supine cycling experiment, with the first measurement 15 seconds into recovery to allow for strain gauge calibration in the original study. The extra blood flow measurement only applied in the context of leg+arm exercise. Following arm-only exercise, only two blood flow measurements were taken, as originally. The exercise protocol is summarised in Fig. 6.1. The whole exercise procedure took 144 minutes.

RESULTS

Terms Used

The results and discussion refer to the three arm exercise workloads as 0 kg, 1 kg, "2" kg, and the 5 bouts of leg exercise as 0% (the arm-only period of exercise), 30%, 60%, 90%, and 30%(2) (the 30% leg intensity repeated again). Each bout of exercise is specifically referred to as the leg intensity x the arm workload, i.e. '60% x "2" kg.'

Heart rate measurements made during the initial leg-only period of exercise are termed HR_{leg}. Those made during the following leg+arm period, or during arm-only exercise are termed HR_{arm}. Heart rate values recorded during the 15 seconds after the arm has finished exercising, but while the legs are still cycling are termed HR_{end}. Blood flow measurements made during this 15 second period are now termed BF1. Those after 15 seconds of rest must thus be termed BF2, and those after 2 minutes of rest are termed BF3. Following arm-only exercise, where only two blood flow measurements are taken, the first after 15 seconds of recovery is termed BF2, and the second after 2 minutes of rest is termed BF3.

Throughout the experiment, the NIRS signal from the exercising forearm was monitored. The measurements of deoxyhaemoglobin, oxyhaemoglobin, and the oxidation state of cytochrome oxidase are termed Hb_{leg}, HbO_{2leg}, and $CtOx_{leg}$ respectively when made from the forearm during the initial leg-only period of

exercise, and Hb_{arm}, HbO_{2arm}, and $CtOx_{arm}$ respectively when made during the arm-only, or leg+arm period. A representative trace from the exercising forearm during the most intensive bout of exercise, at 90% x "2" kg, is shown in Fig. 6.2.

Statistical Analysis

Statistical analysis methods were the same as those adopted in Chapter 3. An analysis of variance using repeated measures with 2 fixed factors was applied to the data initially, with Bonferroni comparison follow-up tests. Further statistical analysis was done by breaking the data down and doing separate 2 way anova tests on the data from each individual arm or leg bout (i.e. looking at the effect of leg intensity on the 1 kg arm workload bouts, or the effect of arm intensity on the 60% leg intensity bouts etc.) again with Bonferroni follow-up comparisons. Simple Students **t** tests were also used where appropriate. Statistical significance was taken at the 5% level and illustrated graphically within groups by an asterisk (*).

Heart Rate

Heart Rate (HR) values were taken at the end of the 1.75 minutes of leg-only exercise just preceding leg+arm exercise (HR_{leg}), again at the end of the arm exercise period (either the arm exercising alone, or superimposed on top of the legs exercising - HR_{arm}), and finally during the last 15 seconds of the 4 minute

leg+arm bouts when the exercising arm had finished activity and its blood flow was being measured whilst the legs were still cycling (HR_{end}).

The HR_{leg} values showed a significant increase with leg intensity (Fig. 6.3). There was also a significant progressive increase in HR_{leg} with exercise bout over the 60% and 90% stages. The HR_{leg} from the final 30%(2) bouts (average = 111.3 ± 3.2 bpm) fell to mid-way between the initial 30% (average = 96.1 ± 2.4 bpm) and the 60% stages (average = 127.2 ± 4.2 bpm). The individual HR_{leg} values from the 30%(2) stages also fell significantly with bout (114.5 ± 3.2 bpm from the first 30%(2) stage, 111.9 ± 3.3 bpm from the second 30%(2) stage, and 107.5 ± 3.0 from the final 30%(2) stage).

The leg+arm stages (HR_{arm} values) produced a similar pattern to the preceding leg-only period (Fig. 6.4). These values again increased significantly with increasing leg intensity, and also with increasing arm workload. A progressive climb in HR_{arm}, similar to that seen in the 60% and 90% HR_{leg} values, is repeated here with the increasing arm workload. The arm exercise must have caused some additional stimulus to heart rate however, as the HR_{arm} values from the arm-only and 30% leg+arm periods also increased significantly with arm workload. The 30%(2) HR_{arm} values, similar to the HR_{leg} values, fell with increasing arm intensity (115.3 ± 4.3 bpm from the 30%(2) x 0 kg stage, 113.1 ± 3.9 bpm from the 30%(2) x 1 kg stage, and 111.9 ± 3.7 from the 30%(2) x "2" kg stage), and overall the 30%(2) HR_{arm} values (average = 113.4 ± 4.0 bpm) again fell to mid-

way between the initial 30% (average = 98.2 ± 2.9 bpm) and the 60% bouts (average = 133.5 ± 4.8 bpm).

The HR_{arm} values showed a slight increase compared to the HR_{leg} values (overall average of 128.6 ± 2.7 bpm in the leg+arm period, compared to 123.3 ± 2.4 bpm in the leg-only period, significant at the p = 0.08 level, Fig. 6.5). This increase of 5.1 bpm compares favourably with the increase of 6.5 bpm over the same comparison in Chapter 3 (Fig. 3.4). The HR_{end} values, taken from the 15 second period after leg+arm exercise in the present study whilst the legs are still cycling and arm blood flow was being taken, are not significantly different from the HR_{arm} values (p = 0.83, Fig. 6.5).

Overall, the heart rate values from the present study are slightly higher, but not significantly different from those seen in Chapter 3 (Table 6.1, Figs. 6.6, 6.7).

Table 6.1: Heart rates during the leg-only, and leg+arm stages of Chapters 3 and 6.

	HR_{leg} (bpm)	HR_{arm} (bpm)
Chapter 3	**118.8** ± 6.6	**125.3** ± 7.4
Chapter 6	**123.5** ± 7.1	**128.6** ± 8.1
Significant Difference	**p = 0.16**	**p = 0.85**

Blood Flow

Blood flow was recorded in the exercising arm after arm exercise whilst the legs were still cycling (BF1), 15 seconds after the complete cessation of exercise (BF2), and 2 minutes after the cessation of exercise (BF3). All values quoted are of blood flow due to exercise, the resting value having been subtracted from each reading. The blood flow values were calculated using the total haemoglobin signal (the deoxyhaemoglobin and oxyhaemoglobin signals) from the NIRS measurements. This was converted into blood flow values by using an average haemoglobin concentration of 15 $g.dl^{-1}$ in the blood.

BF1 showed a general increase with increasing arm intensity (Fig. 6.8). The 1 kg and "2" kg arm workloads did not differ significantly at any of the leg intensities. Both were significantly greater than the 0 kg arm workload, except in the 90% bouts, when there was no significant difference due to any of the arm intensities. BF1 also increased with leg intensity, particularly evident in the 0 kg bouts (Fig. 6.9). It continued to increase through the final 30%(2) bouts, giving the highest BF values of the study in the 30%(2) x 1 kg (111.9 ± 22.1 $ml.100ml^{-1}.min^{-1}$) and 30%(2) x "2" kg bouts (111.9 ± 21.5 $ml.100ml^{-1}.min^{-1}$). Differences in the BF1 values become less apparent with increasing arm workload. In the "2" kg bouts, there was no significant difference between the different leg intensities.

The BF2 values, taken 15 seconds after the end of exercise (30 seconds after the end of arm exercise) show a much clearer increase with increasing arm workload

(Fig. 6.10). The BF2 values became significantly greater with increasing arm workload throughout the range of leg exercise intensities. Again, blood flow increased with increasing leg intensity, but only from the 60% leg exercise bouts onwards (Fig. 6.11). The higher leg exercise intensities (60%, 90%), and the last leg workload (30%(2)) generally produced values that were not significantly different, but higher than the 0% and 30% values.

The BF2 values were generally all less than the BF1 values, except in the 90% leg intensity bouts (Fig. 6.12). The increasing arm exercise intensity seemed unable to cause an increase in the BF1 values during 90% leg exercise (Fig. 6.8). In the 90% x "2" kg bouts, the BF2 value (102.5 ± 15.0 $ml.100ml^{-1}.min^{-1}$) actually increases from the BF1 value (70.7 ± 21.6 $ml.100ml^{-1}.min^{-1}$), significant at a level of $p = 0.12$. The hyperaemia is also maintained in the BF3 readings (Fig. 6.12). The BF3 values show the same general pattern of increasing significantly with increasing arm and leg intensity as seen in the BF1 and BF2 values. In the 90% x "2" kg bout the average BF3 value (98.9 ± 12.6 $ml.100ml^{-1}.min^{-1}$) is not significantly different to the BF2 value, but is higher than the BF1 value at a significance level of $p = 0.13$.

The pattern of blood flow change with changing exercise bout was very similar between BF2 and BF3 of the present study, and BF1 and BF2 of Chapter 3 (Fig. 6.13). However, the values obtained in the present study seem approximately 4 times higher.

Near Infrared Spectroscopy

The NIRS variables from the forearm during the 1.75 minute period of leg-only exercise (termed Hb_{leg}, HbO_{2leg}, $CtOx_{leg}$) were analysed separately from the 2 minute period of arm-only, or leg+arm exercise (termed Hb_{arm}, HbO_{2arm}, $CtOx_{arm}$), blood flow being analysed from data in the remaining 15 second period. As before, the difference between the averages of 4-second samples of data from the beginning and end of the analysis period was calculated, then divided by the time between in minutes, to get the rate of change in $\mu mol.min^{-1}$. In the case of the 2 minute periods of arm-only and leg+arm exercise, previous procedures were again followed, and it was further split down into the change in the first 30 seconds of exercise, and the change in the remaining 1.5 minutes. In these calculations, the same 4-second period of data averaged for the end of the initial 30 second interval was used as the start of the latter 1.5 minute interval.

Deoxyhaemoglobin (Hb), Oxyhaemoglobin (HbO_2)

During the initial leg-only period of exercise, the Hb values from the forearm generally increased and the HbO_2 values generally fell, but very little change was seen between the 3 bouts at each leg workload (Hb_{leg}, HbO_{2leg}; Figs. 6.14, 6.15). However, during the 90% of maximum leg intensity workloads, there was a significantly greater rate of Hb_{leg} increase, and HbO_{2leg} decrease. The greatest rate

of Hb_{leg} increase was seen in the second 90% bout (6.7 ± 1.1 $\mu mol.min^{-1}$), and the greatest rate of HbO_{2leg} fall was in the first 90% bout (12.1 ± 1.6 $\mu mol.min^{-1}$).

In the arm-only and leg+arm periods of exercise, a significant effect on the Hb and HbO_2 signals in the exercising forearm was seen due to arm exercise intensity. The rate of Hb_{arm} increase became significantly greater with increasing arm intensity over the 2 minute exercise period (Fig. 6.16), the maximum rate of change occurring in the 30% x "2" kg bout (20.9 ± 2.2 $\mu mol.min^{-1}$). The HbO_{2arm} signal became significantly lower with increasing exercising arm intensity (Fig. 6.17), with maximum rate of decline occurring in the 30%(2) x "2" kg bout (7.8 ± 1.6 $\mu mol.min^{-1}$). However, the change was not as clearly significant as was the case with the Hb_{arm} values, only isolated "2" kg bouts showing any significant difference at each leg exercise intensity. In the 90% leg exercise bouts however, the rate of change of HbO_{2arm} from the 0 kg arm workload showed the greatest fall, significantly greater than the rate of change seen during the 90% x 1 kg bout. Both the Hb_{arm} and the HbO_{2arm} values showed slightly smaller rates of change during the maximum 90% x "2" kg bouts than during the other "2" kg bouts.

The data from the arm-only and the leg+arm periods of exercise were then further broken down into the first 30 seconds and the last 1.5 minutes. The Hb_{arm} data still showed the same clearly significant greater rate of increase with increasing arm workload when analysed over the first 30 seconds (Fig. 6.18), but the HbO_{2arm} data now also showed a clear pattern, falling significantly with increasing arm workload (Fig. 6.20). Very little change occurred in the Hb_{arm} data in the last

1.5 minutes of leg+arm or arm-only exercise (Fig. 6.19). The HbO_{2arm} however, generally recovered somewhat in the last 1.5 minutes of each 1 kg, or "2" kg bout (Fig. 6.21), being not significantly different from each other, but greater than the 0 kg bouts.

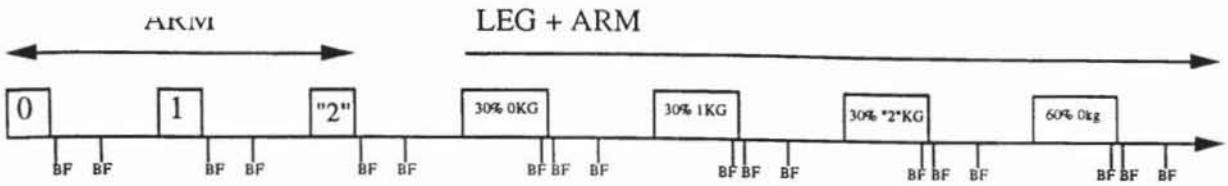
Cytochrome Oxidase (CtOx)

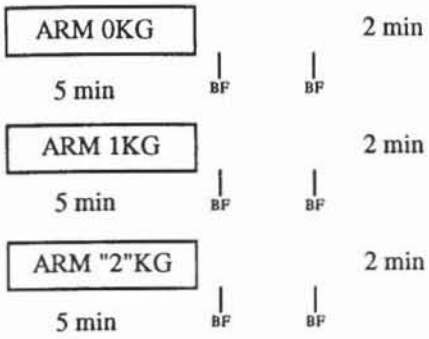
Little change was seen in the cytochrome oxidase oxidation state in the exercising forearm muscles during the leg-only periods of exercise ($CtOx_{leg}$, Fig. 6.22). The cytochrome oxidase enzyme became reduced most rapidly during the first 30%(2) stage (0.5 ± 0.4 $\mu mol.min^{-1}$). During the third bouts at the 90% and 30%(2) leg intensities, the enzyme became significantly more oxidised/less reduced than in the earlier bouts at the respective leg workload.

During the arm-only and leg+arm exercise periods, the $CtOx_{arm}$ redox state showed little change during the 0 kg arm workloads, but became significantly reduced over the 2 minutes of 1 kg and "2" kg exercise (Fig. 6.23). This reached a peak during the 30% x "2" kg bout (0.7 ± 0.2 $\mu mol.min^{-1}$). There was however, very little effect of increasing leg intensity.

The greatest rate of change in the cytochrome oxidase oxidation state occurred in the first 30 seconds of the arm-only and leg+arm bouts (Fig. 6.24). In the last 1.5 minutes, the cytochrome oxidase enzyme kept the same general pattern of

becoming more reduced with increasing arm workload as seen in the first 30 seconds of exercise, and that seen overall (Fig. 6.23), but were generally slightly more oxidised compared to the first 30 seconds (Fig. 6.25).





BF - Blood Flow Measurement

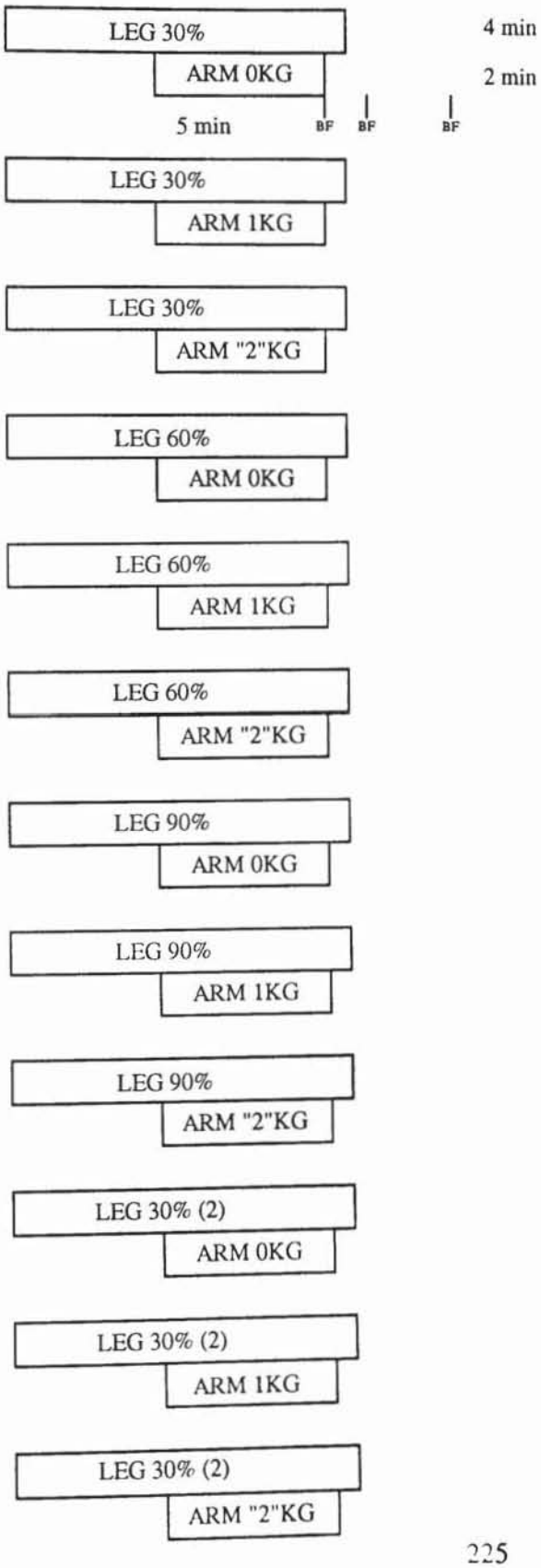


Fig. 6.1
Protocol used when superimposing arm exercise upon leg exercise, when using NIRS analysis (Chapter 6).

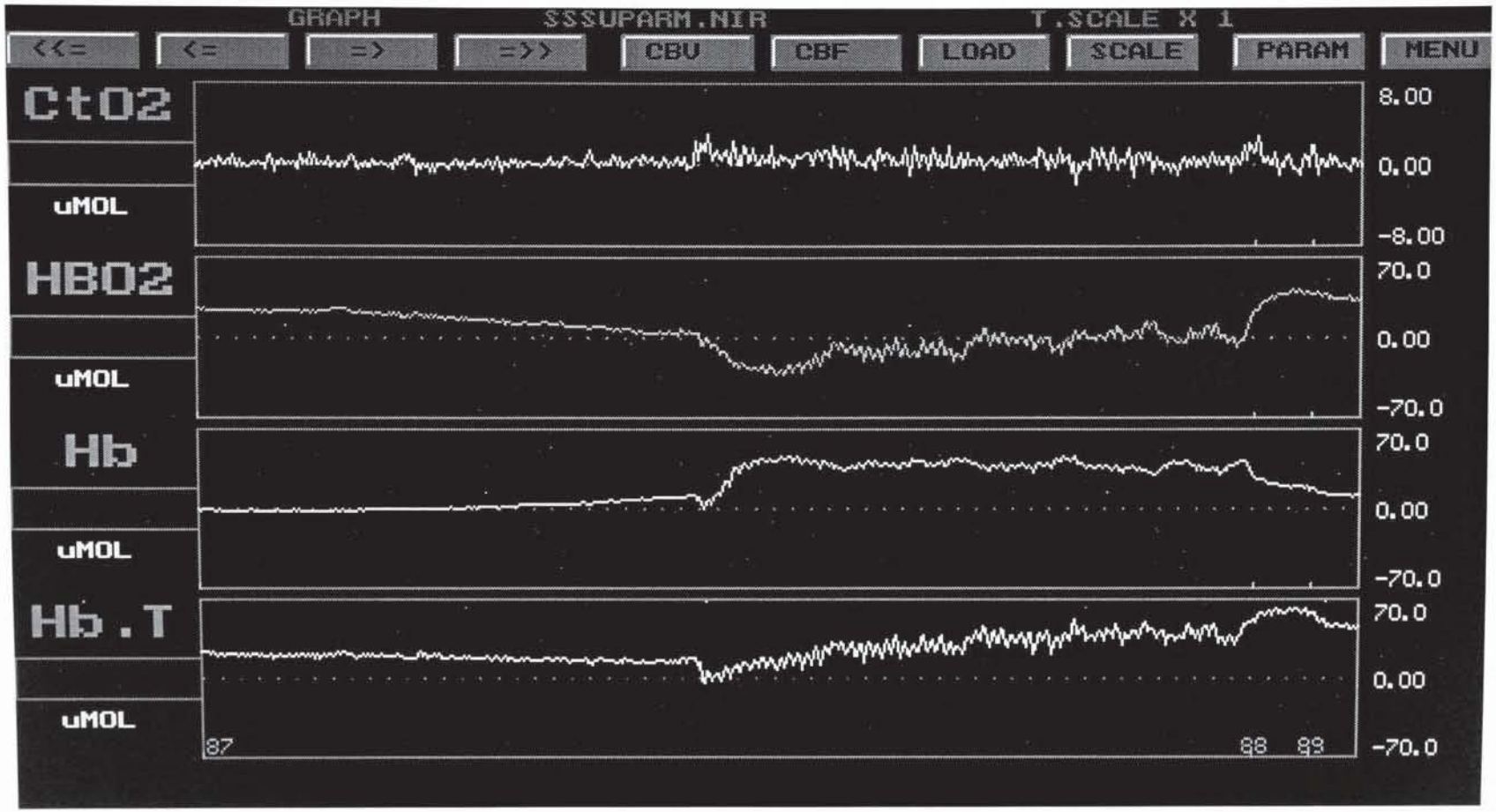


Fig. 6.2: Trace showing 1.75 minute period of leg-only exercise (from event 87), 2 minute period of leg+arm exercise (ending event 88), and 15 second period of leg-only exercise again, during venous occlusion blood flow measurement (ending event 89). Headings on left refer to oxidised cytochrome oxidase (CtO2), Oxyhaemoglobin (HbO2), Deoxyhaemoglobin (Hb), and Total Haemoglobin (Hb.T [= HbO2 + Hb]). Values on right are full scale deflection concentration changes in mM, from zero set at rest.

Fig. 6.3

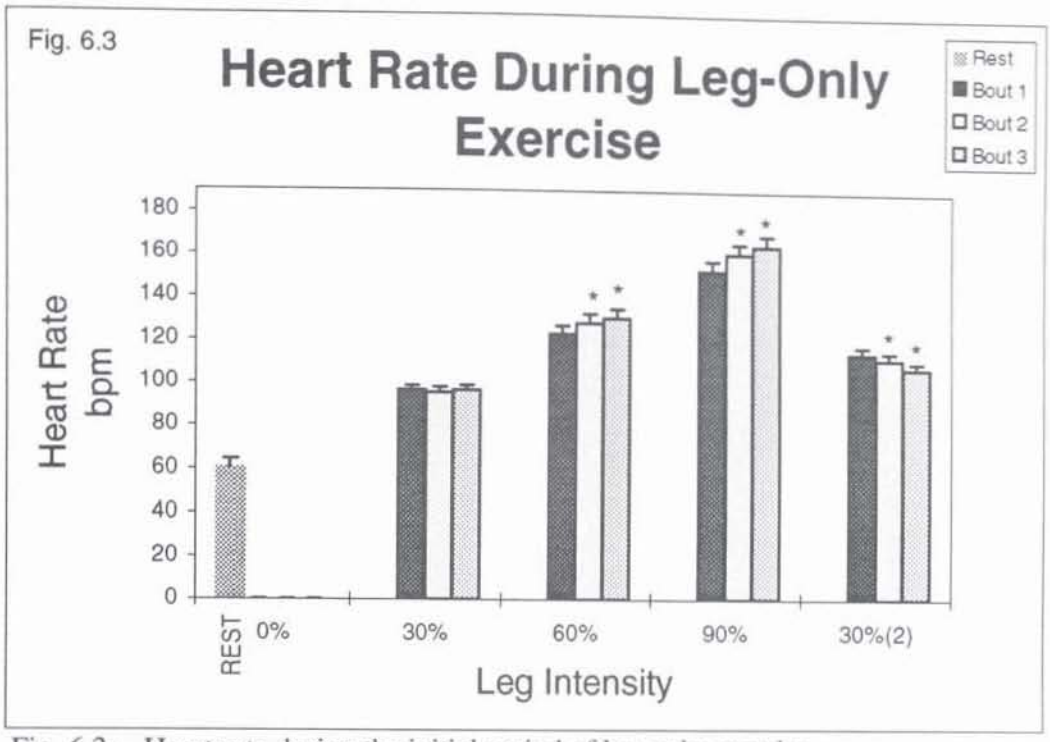


Fig. 6.3: Heart rate during the initial period of leg-only exercise.

Fig. 6.4

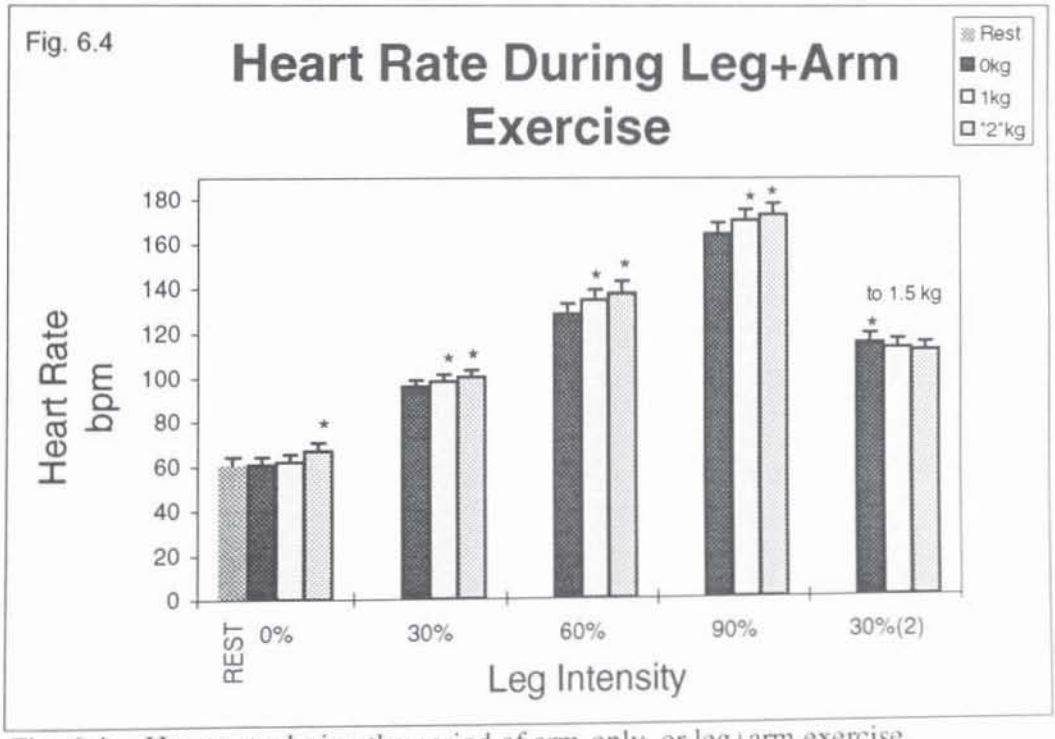


Fig. 6.4: Heart rate during the period of arm-only, or leg+arm exercise.

Fig. 6.5

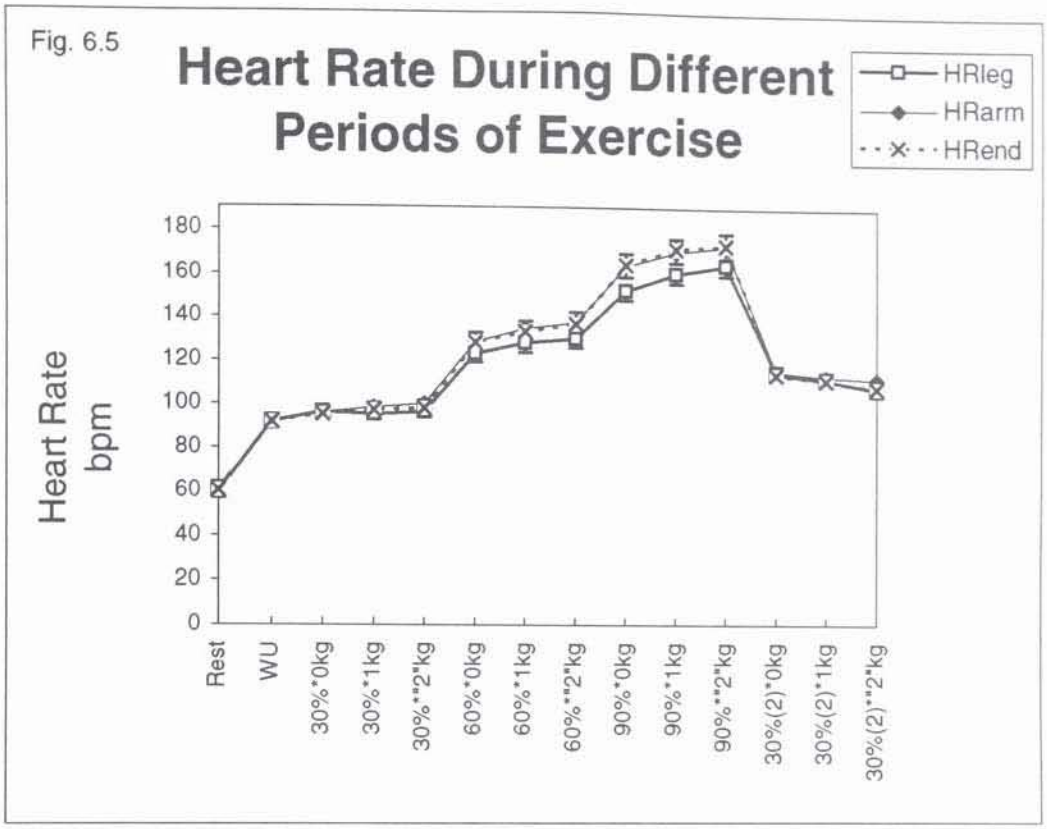


Fig. 6.5: Heart rate during initial leg-only exercise, leg+arm exercise, and the last 15 seconds, when the legs continue to cycle but the arm exercise has ended.

Fig. 6.6

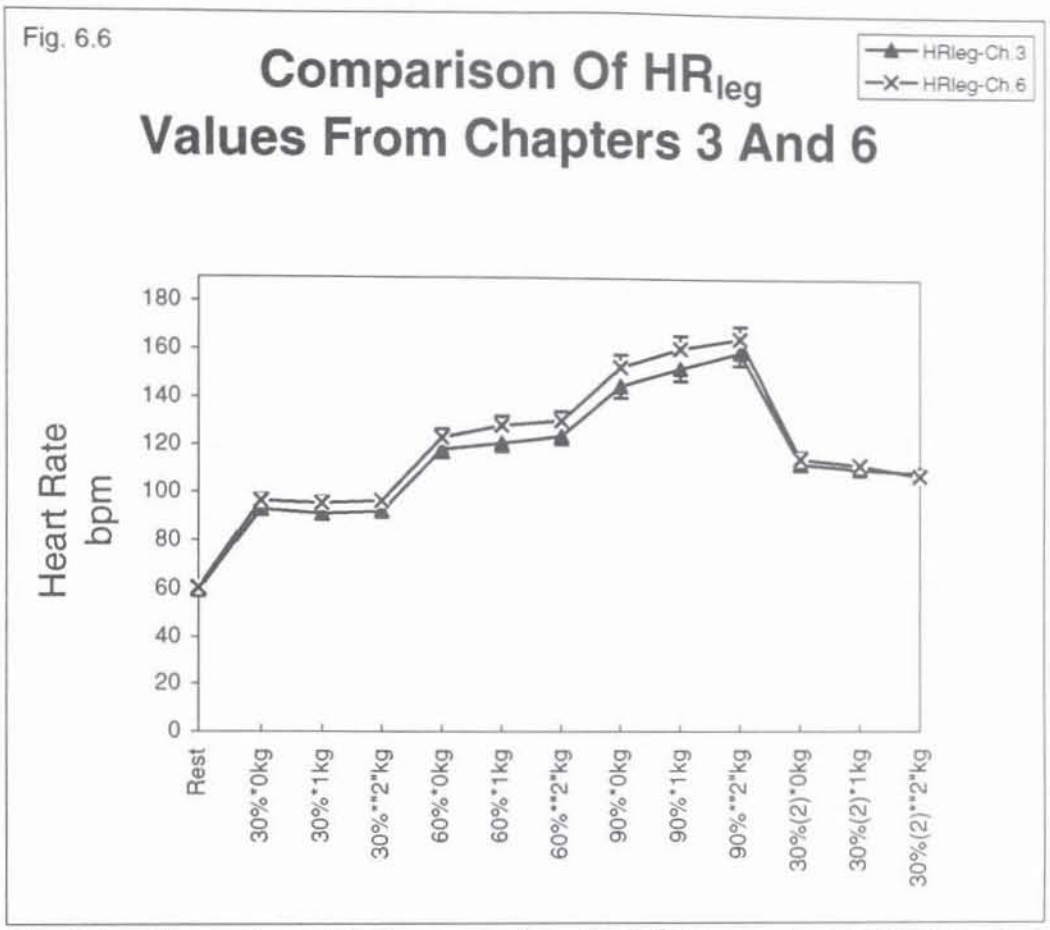


Fig. 6.6: Comparison of the heart rates from the initial leg-only periods of exercise from the present study and that of Chapter 3.

Fig. 6.7

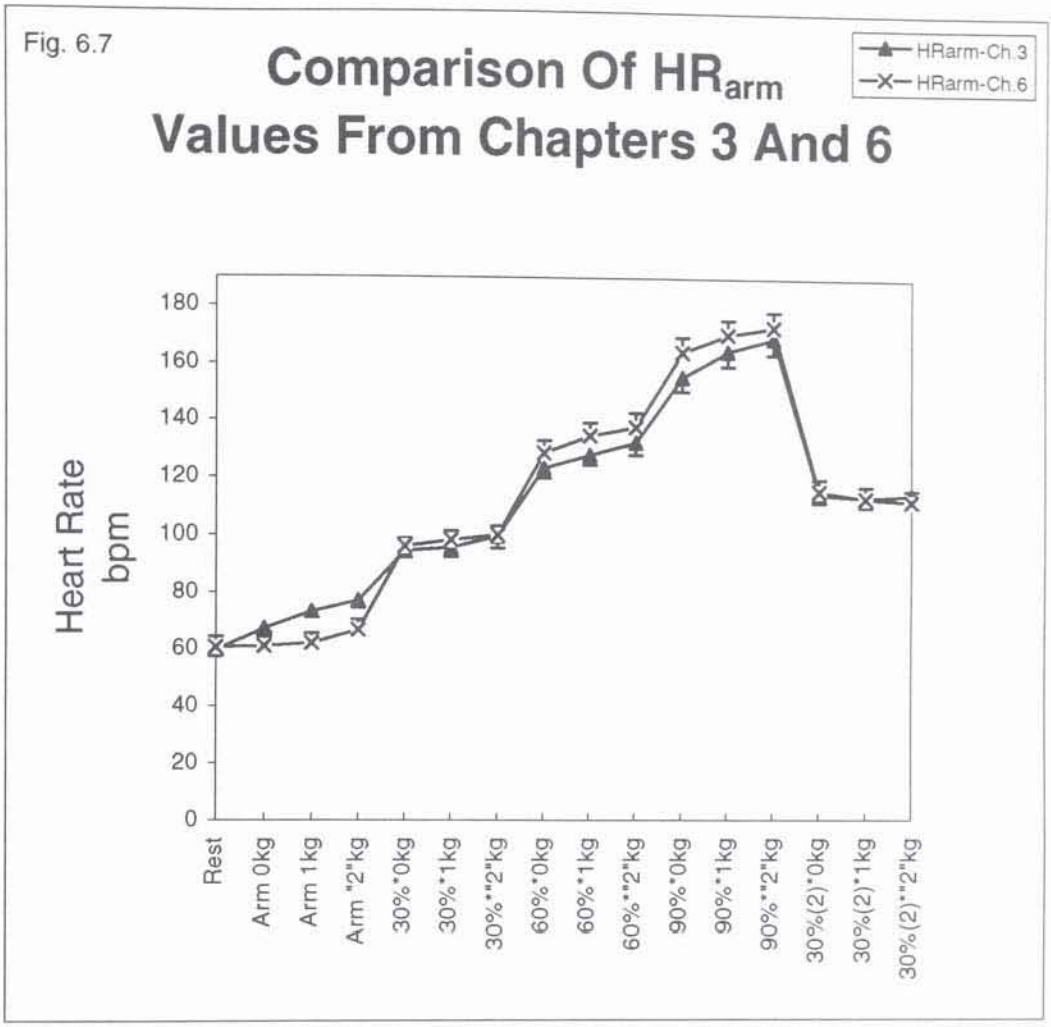


Fig. 6.7: Comparison of the heart rates from the arm-only and the leg+arm periods of exercise from the present study and that of Chapter 3.

Fig. 6.8

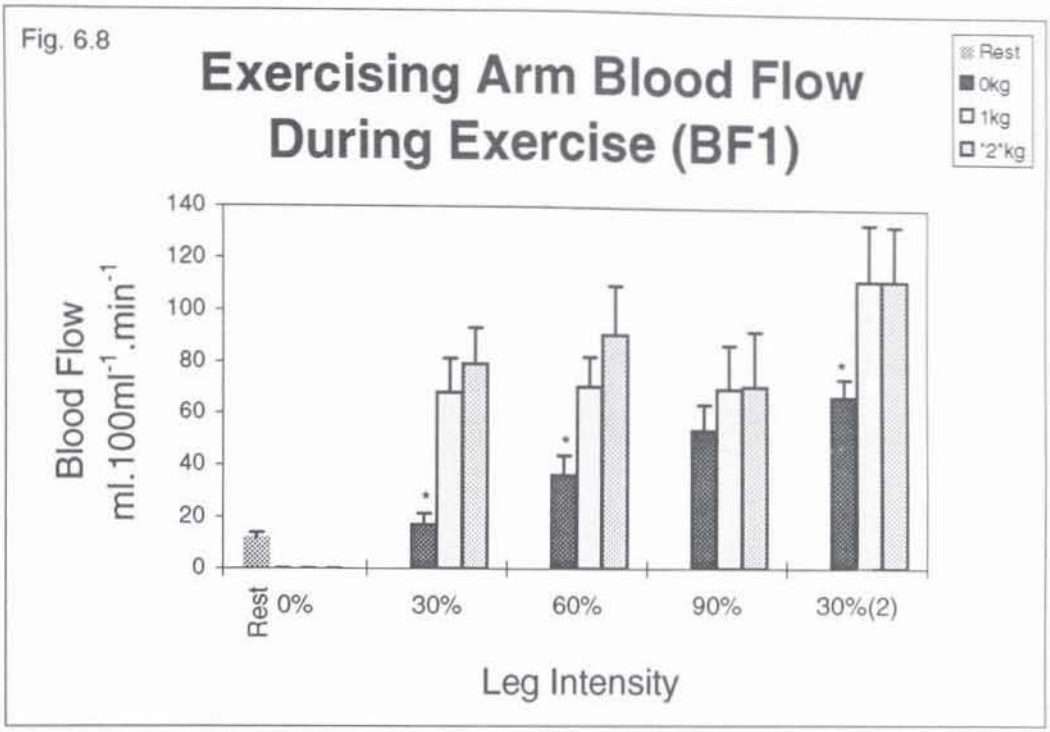


Fig. 6.8: Exercising arm blood flow taken while the legs are still cycling (BF1), plotted in relation to leg exercise intensity.

Fig. 6.9

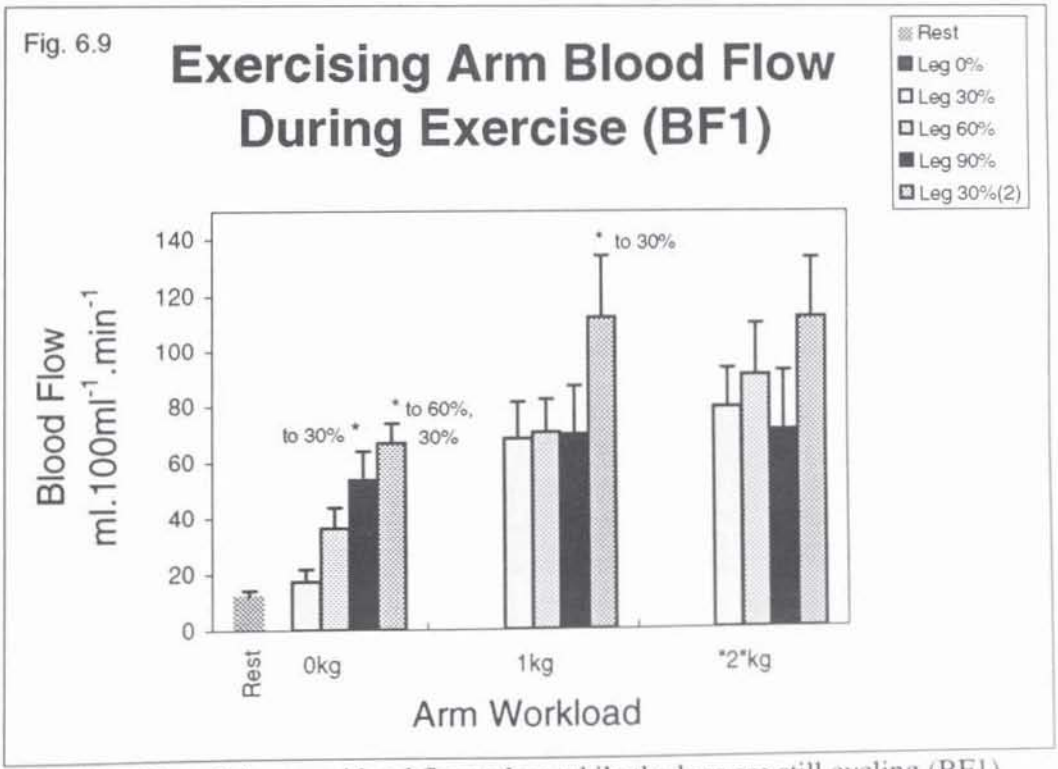


Fig. 6.9: Exercising arm blood flow taken while the legs are still cycling (BF1), plotted in relation to arm workload.

Fig. 6.10

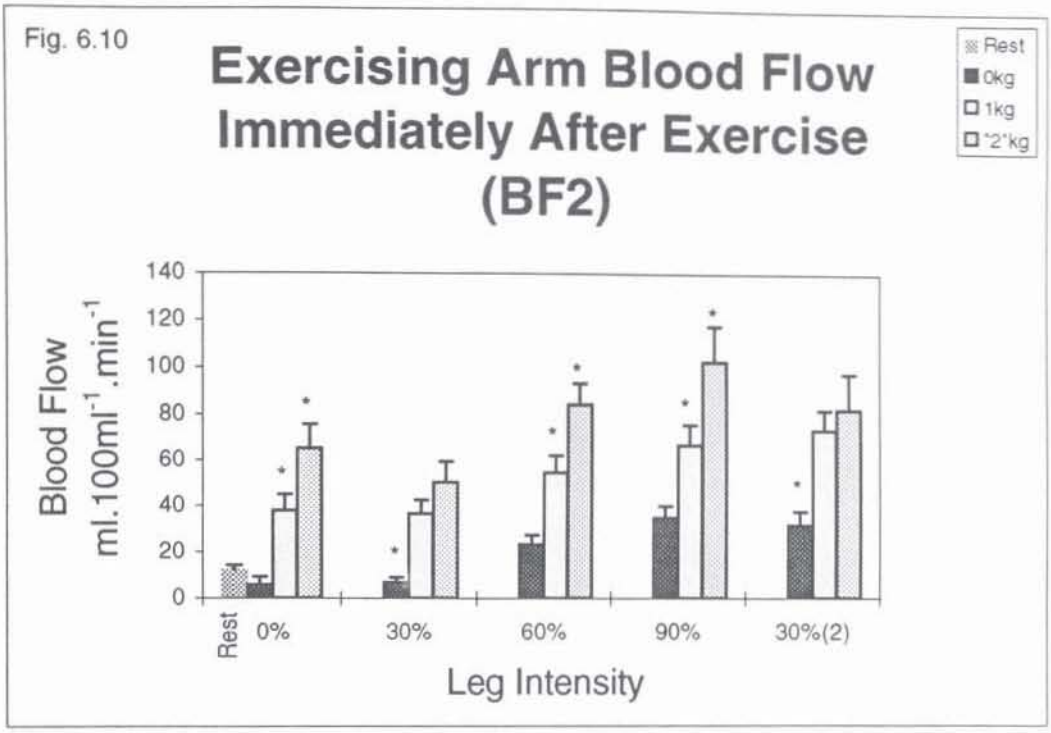


Fig. 6.10: Exercising arm blood flow 15 seconds after the end of exercise (BF2), plotted in relation to leg exercise intensity.

Fig. 6.11

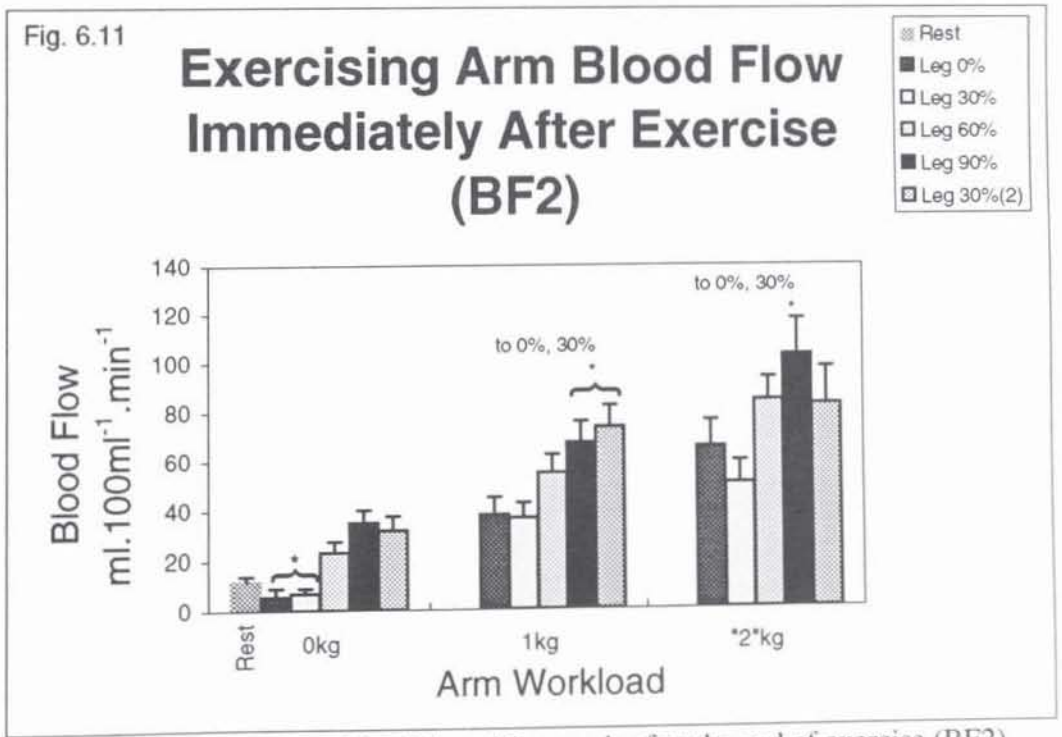


Fig. 6.11: Exercising arm blood flow 15 seconds after the end of exercise (BF2), plotted in relation to arm workload.

Fig. 6.12

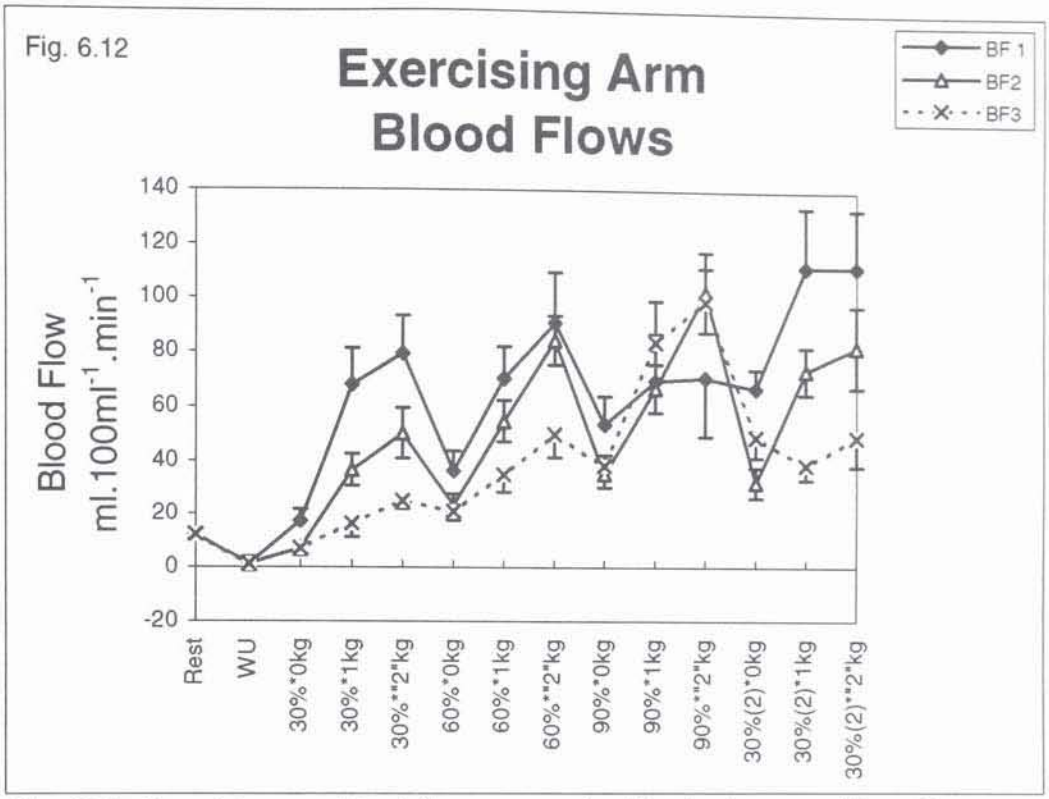


Fig. 6.12: Exercising arm blood flow measured whilst the legs are still cycling (BF1), 15 seconds after the end of exercise (BF2), and 2 minutes after the end of exercise (BF3).

Fig. 6.13

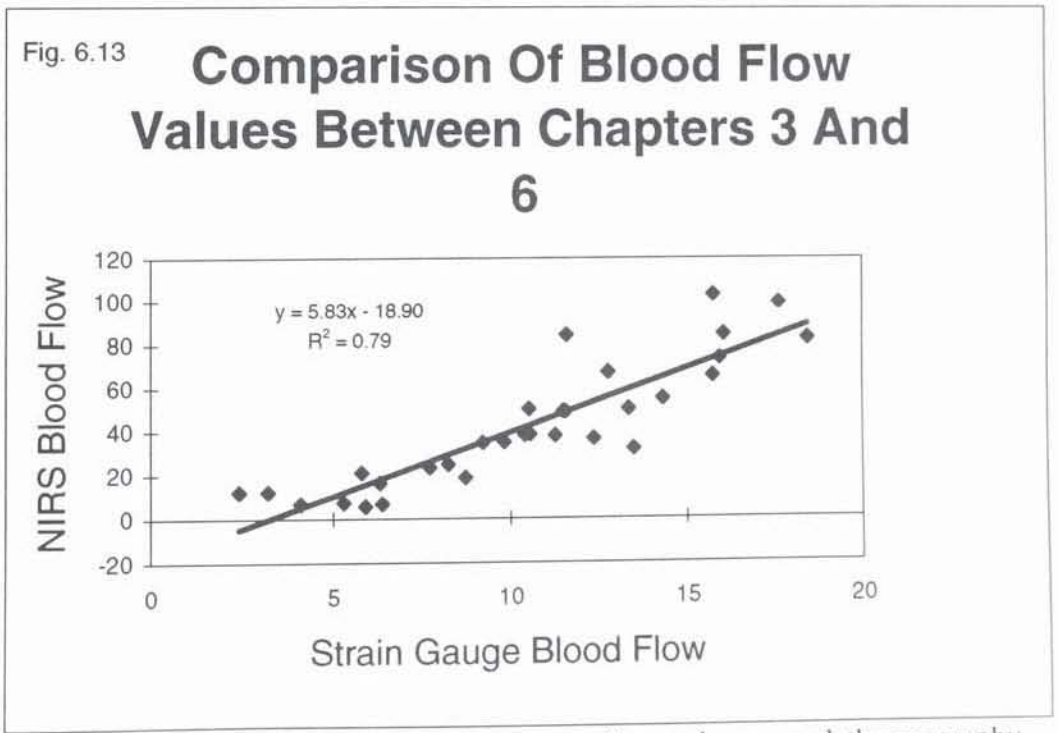


Fig. 6.13: Comparison of blood flows measured by strain gauge plethysmography (Chapter 3), and by the total haemoglobin signal (Hb + HbO_2) from NIRS. All blood flows in $ml.100ml^{-1}.min^{-1}$.

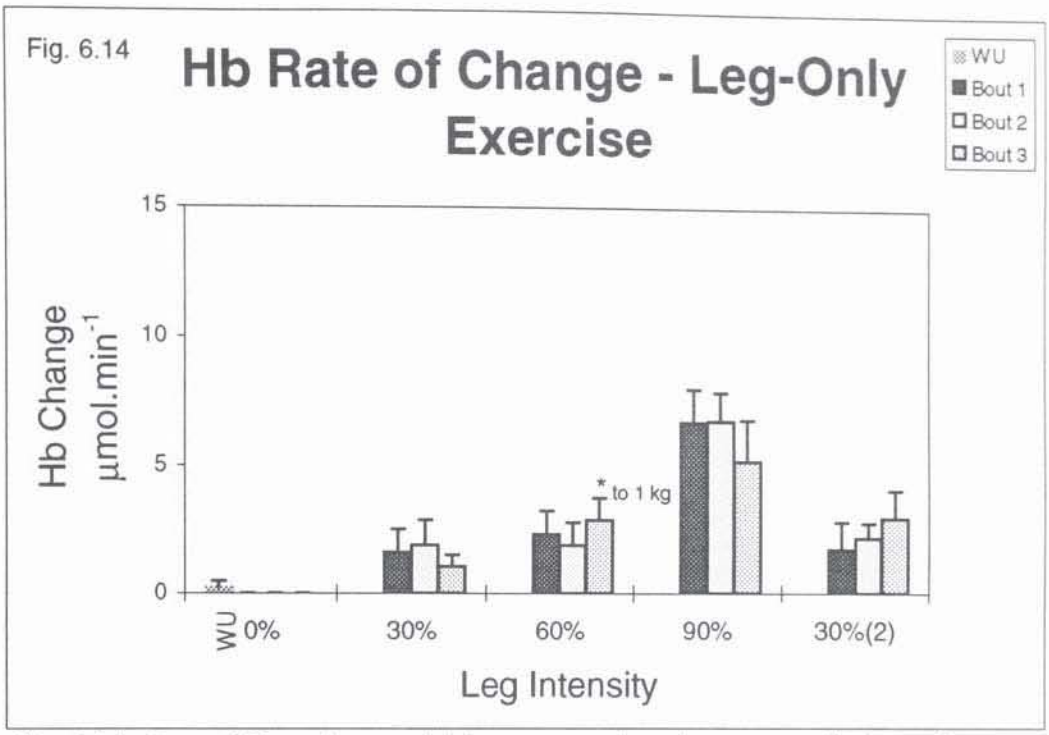


Fig. 6.14: Rate of Deoxyhaemoglobin concentration change over the leg-only period of exercise.

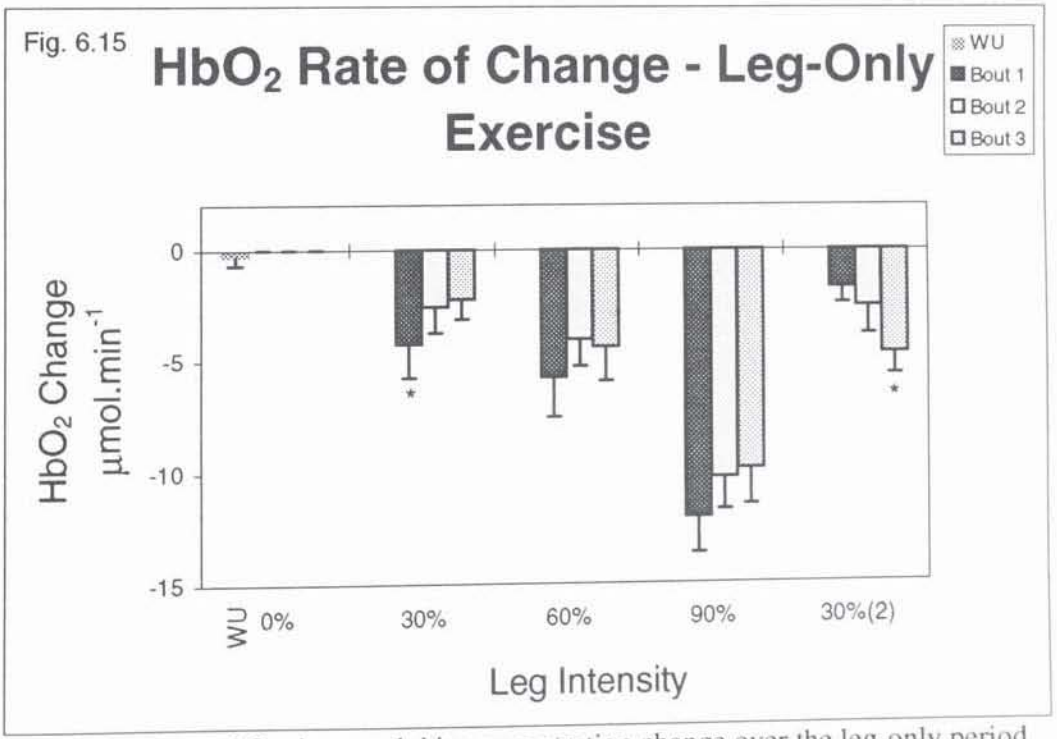


Fig. 6.15: Rate of Oxyhaemoglobin concentration change over the leg-only period of exercise.

Fig. 6.16

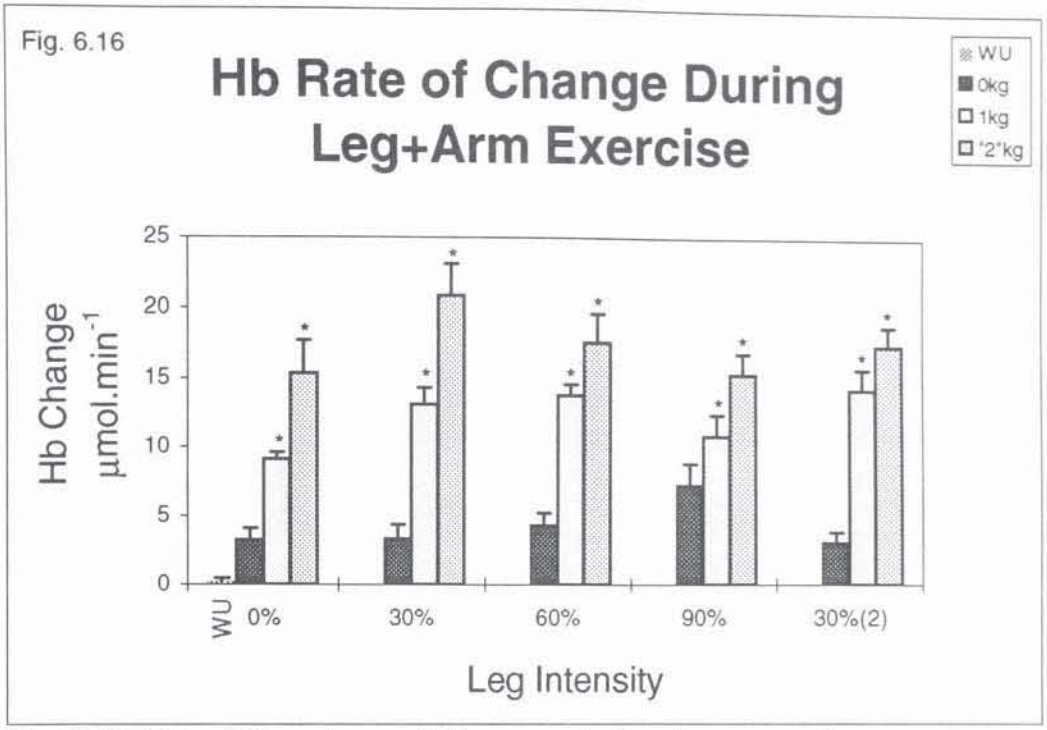


Fig. 6.16: Rate of Deoxyhaemoglobin concentration change over the arm-only, or the leg+arm period of exercise.

Fig. 6.17

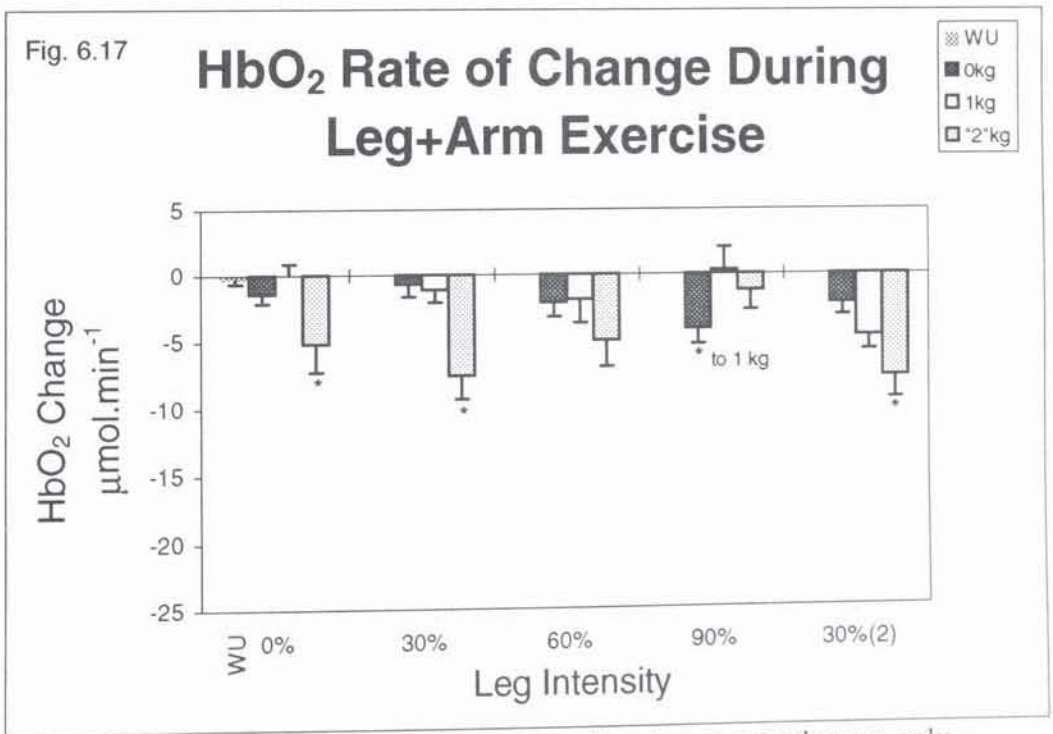


Fig. 6.17: Rate of Oxyhaemoglobin concentration change over the arm-only, or the leg+arm period of exercise.

Fig. 6.18

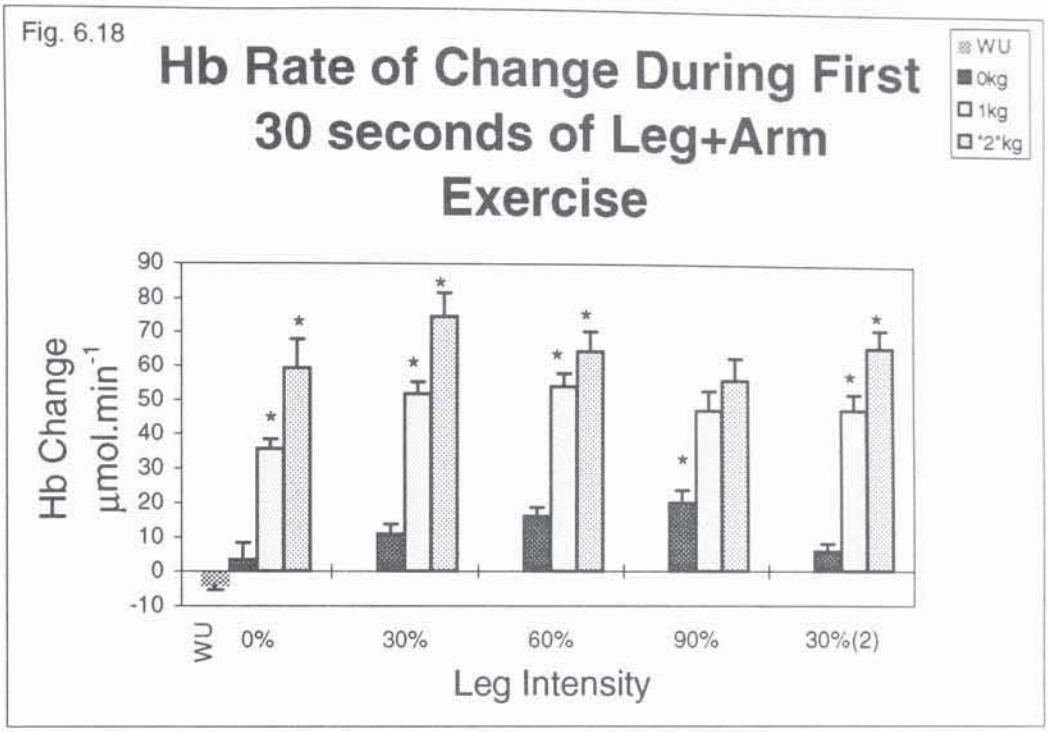


Fig. 6.18: Deoxyhaemoglobin rate of change during the first 30 seconds of the 2 minute period of arm-only, or leg+arm exercise.

Fig. 6.19

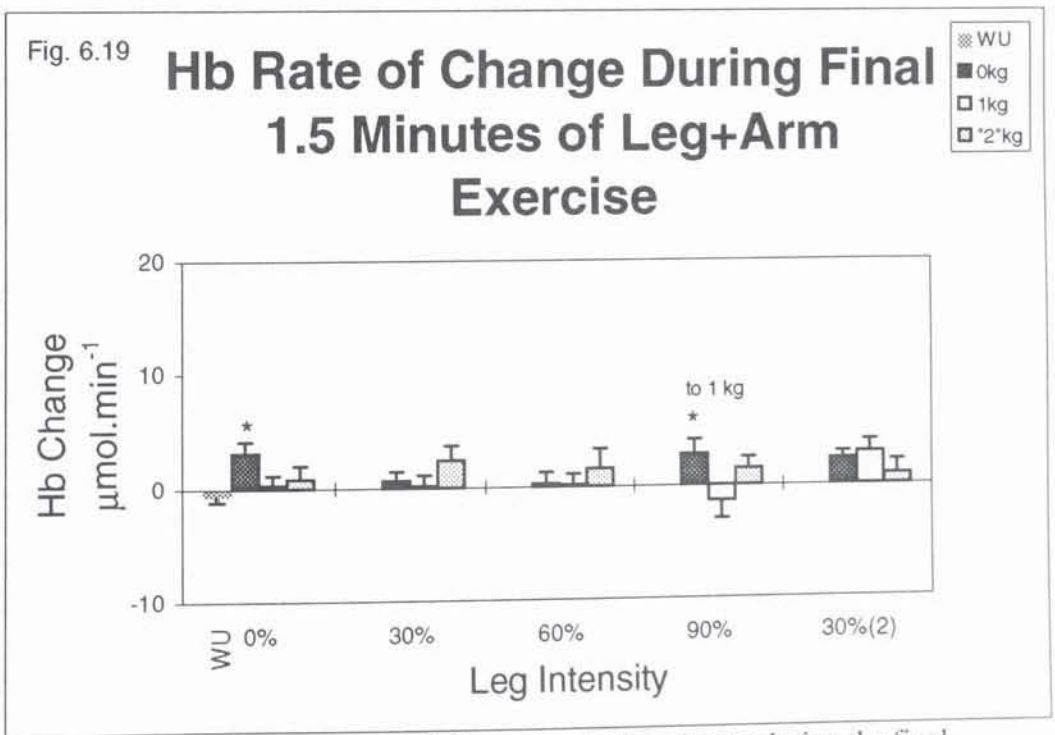


Fig. 6.19: Rate of Deoxyhaemoglobin concentration change during the final 1.5 minutes of the 2 minute period of arm-only, or leg+arm exercise.

Fig. 6.20

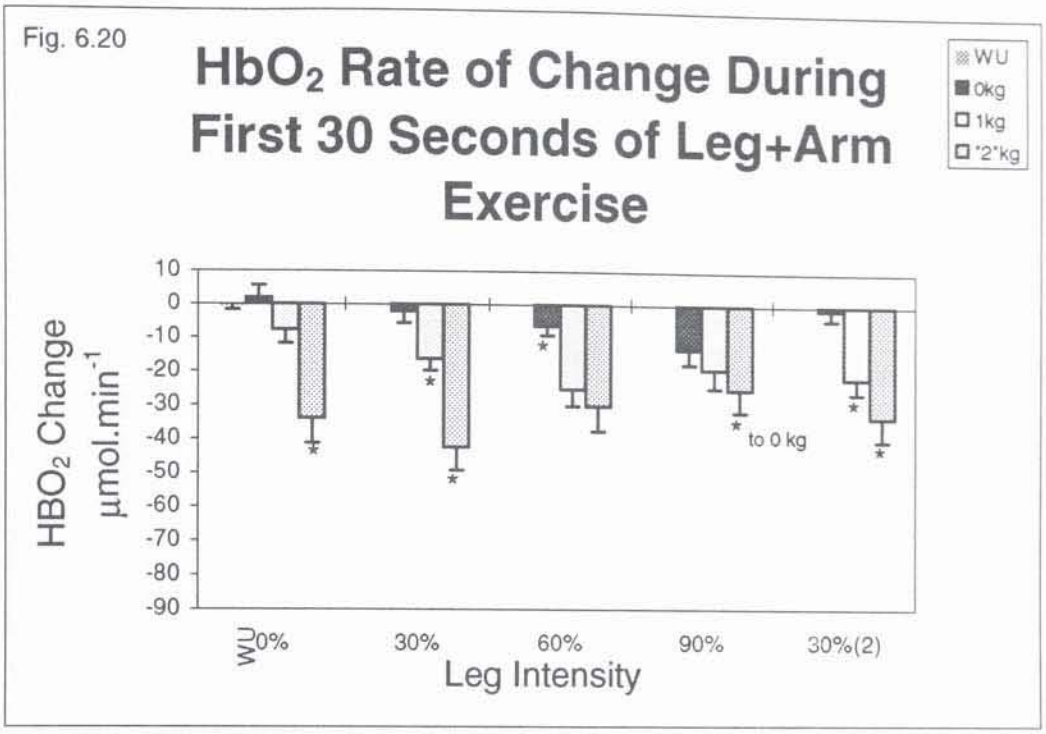


Fig. 6.20: Rate of Oxyhaemoglobin concentration change during the first 30 seconds of the 2 minute period of arm-only, or leg+arm exercise.

Fig. 6.21

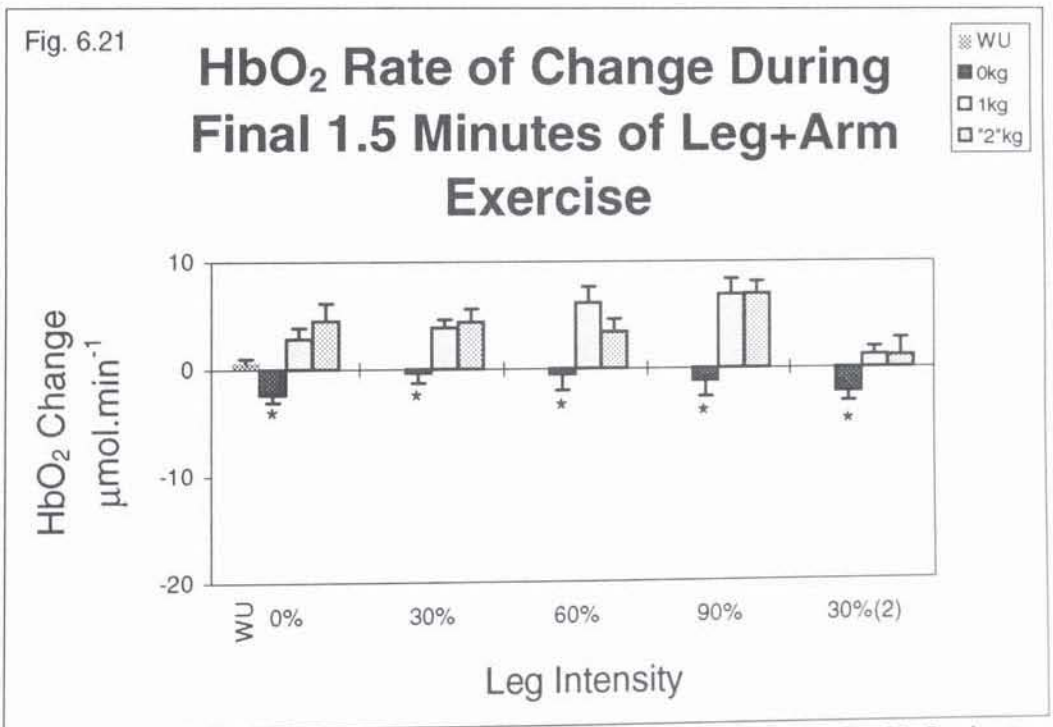


Fig. 6.21: Rate of Oxyhaemoglobin concentration change over the final 1.5 minutes of the 2 minute period of arm-only, or leg+arm exercise.

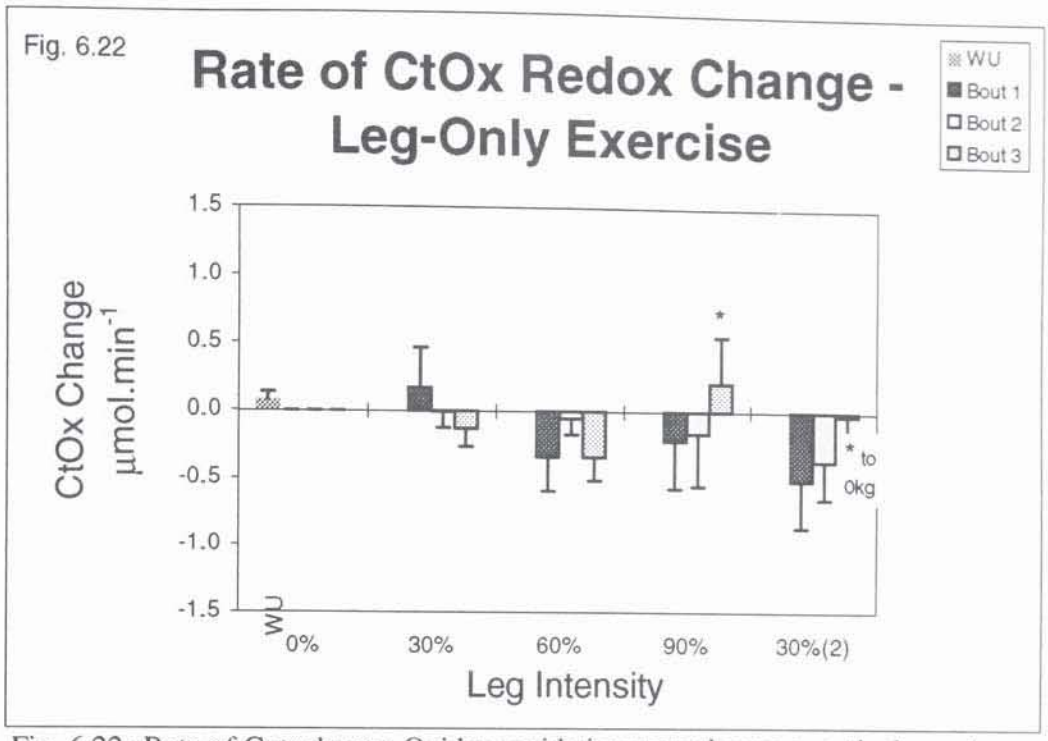


Fig. 6.22: Rate of Cytochrome Oxidase oxidation state change over the leg-only period of exercise.

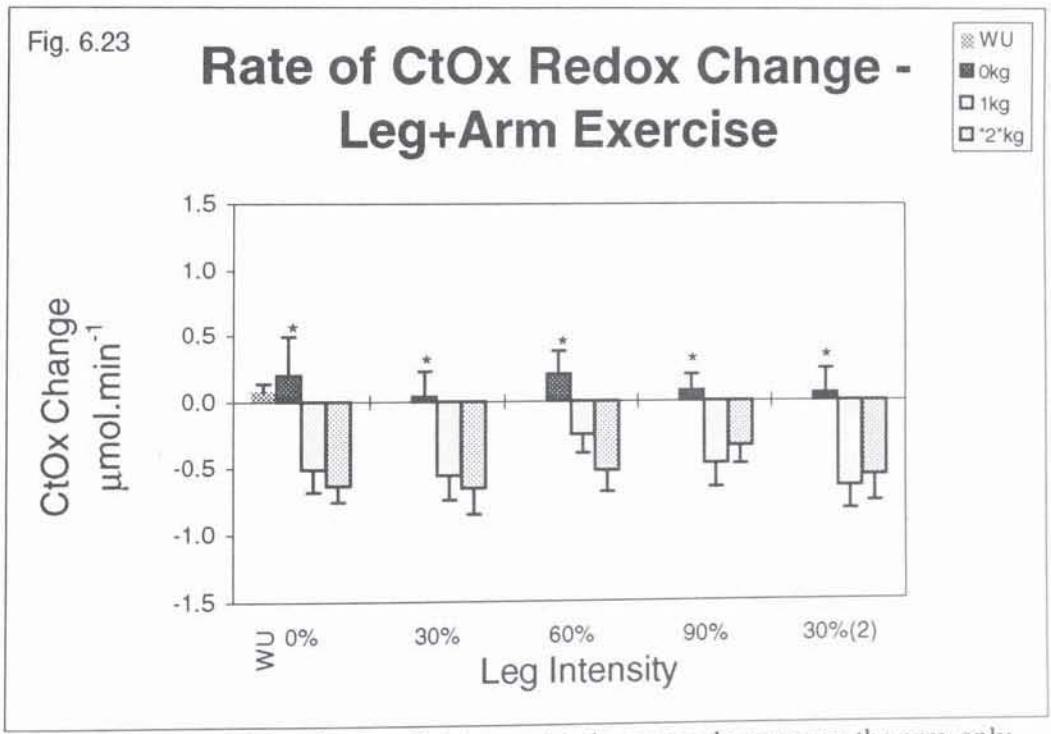


Fig. 6.23: Rate of Cytochrome Oxidase oxidation state change over the arm-only, of leg+arm periods of exercise.

Fig. 6.24

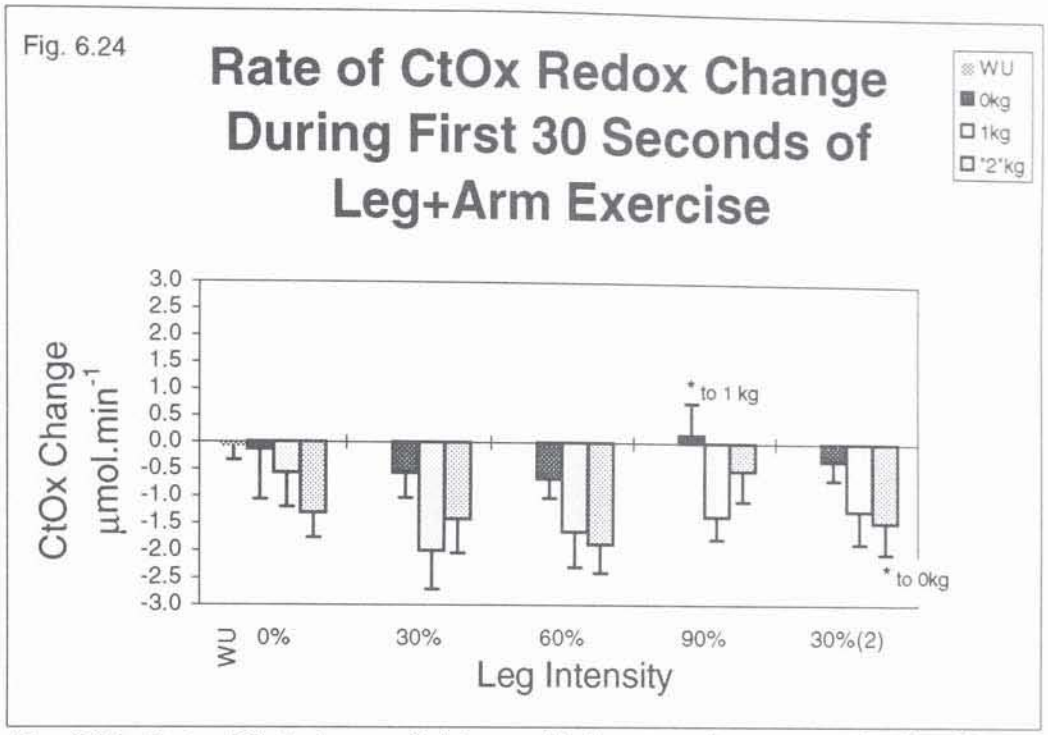


Fig. 6.24: Rate of Cytochrome Oxidase oxidation state change over the first 30 seconds of the 2 minute period of arm-only, or leg+arm exercise.

Fig. 6.25

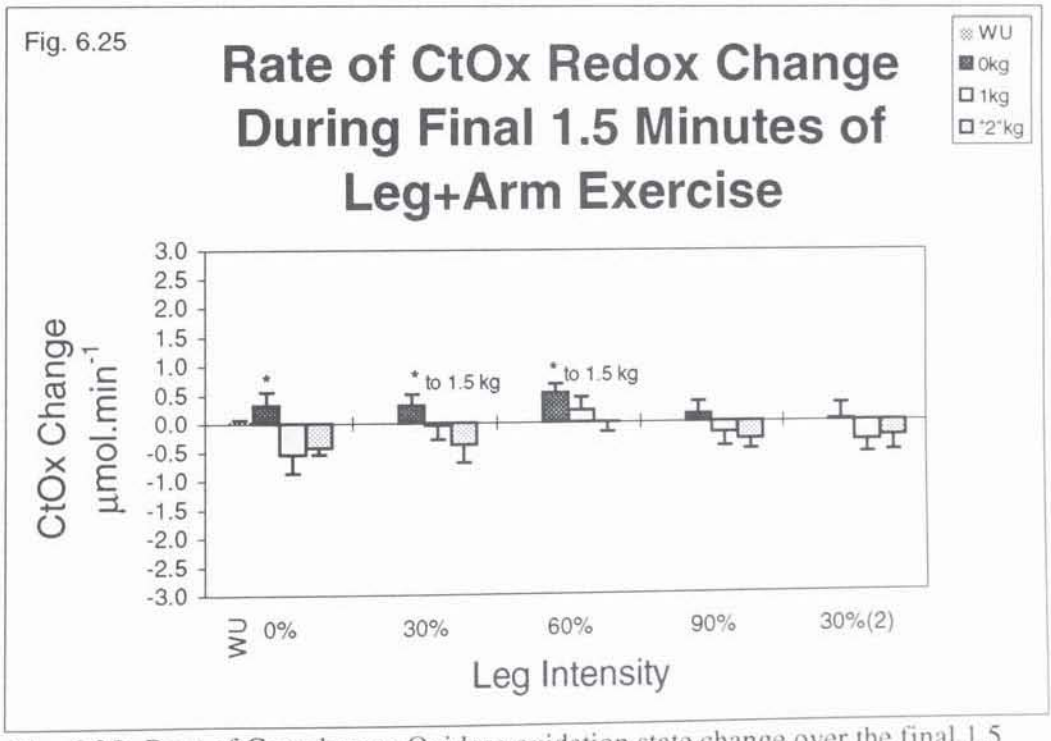


Fig. 6.25: Rate of Cytochrome Oxidase oxidation state change over the final 1.5 minutes of the 2 minute period of arm-only, or leg+arm exercise.

DISCUSSION

Heart Rate

The heart rate results from this study are largely similar to those obtained in the first supine cycling study (Table 6.1, Figs. 6.6, 6.7). The present study, although not significantly different, did produce heart rates that were slightly higher than the equivalent period of Chapter 3, mainly during the leg-only periods. This may be partly due to the slightly different modes of action of the two ergometers. The Siemens electronically braked ergometer allowed a period of 20-25 seconds at the start of exercise, in which the load was gradually built up to the required level. This period was absent in the present study, although the initial leg only period was 15 seconds shorter at 1.75 minutes. Another contributing factor may be due to the unfortunate inability to verify the calibration of the Siemens ergometer. The two ergometers produced vary similar heart rate values so were very close in the loads they applied, but they may not have been identical.

In both studies the heart rate values increased from leg-only, to leg+arm exercise at each workload (Table 6.1). The average increases in Chapter 3 due to adding the arm exercise was 6.5 bpm, and was very close to the increase of 5.1 bpm seen in the present study. This apparent disproportionately large increase from adding 0.2-0.25 kg of exercising forearm muscle to 14-16 kg of exercising leg muscle can again be attributed to an increased isometric component of upper body exercise.

less skeletal muscle pump activity possibly affecting venous return, a more dominating sympathetic tone during arm exercise (Christensen, 1931; Åstrand *et al.*, 1968; Toner *et al.*, 1983), and perhaps a small cardiovascular drift effect at the more intensive leg intensities (Rowell, 1993a), as outlined in Chapter 3 (p. 97).

The HR_{end} values, taken from the final 15 seconds of the 4 minute exercise bout, when the legs were still cycling, but the arm had stopped, were not significantly different from the preceding HR_{arm} values. The same whole body cardiovascular challenge can therefore be assumed to be occurring in the two periods. The blood flow, measured during the final 15 seconds, can be applied with some justification to the end of the leg+arm exercise period. However, some blood flow redistribution may be argued to be occurring between the now resting arm and the still exercising legs. The limitations of the present experiment prevented a closer approximation to exercising arm blood flow.

Blood Flow

Blood flow was measured by the increase in the total (oxy- plus deoxy-) haemoglobin concentration under venous occlusion. The pattern of change due to the leg and arm workloads in the present study was very similar to the pattern of blood flow estimated by strain gauge plethysmography in Chapter 3 (BF1 and BF2 in the original study, compared to BF2 and BF3 in the present investigation

respectively - Fig. 6.13). On application of venous occlusion, blood collecting in the forearm may alter the tissue optical properties, by causing a change in scattering. However, the level of optical scattering has been shown to change by less than 7% after 3 minutes of venous occlusion (Ferrari *et al.*, 1992). Negligible interference was therefore concluded to be occurring over the 10-15 second occlusion period used in the present study.

The blood flow values calculated from the NIR data were, however, approximately 4 times higher than those obtained from strain-gauge plethysmography. NIR light penetration is dependant on the optode separation. At a 4 cm interoptode spacing, the depth of penetration will also be approximately 4 cm (Cui *et al.*, 1991). The NIRS signals will be dominated by the intramuscular conditions. Blood flow in the muscle has been shown to be capable of reaching up to 200 $ml.100ml^{-1}.min^{-1}$ in exercise, with peak flows of 300 $ml.100ml^{-1}.min^{-1}$ possible (Andersen & Saltin, 1985; Saltin, 1986; Rowell *et al.*, 1986; Waaler *et al.*, 1987; Walloe & Wesche, 1988). Blood flow measured by venous occlusion plethysmography must underestimate the actual muscle blood flow measurements by incorporating other relatively avascular tissues, non-working muscles, and relatively vascular tissues that are comparatively unaffected by immediate local exercise, in addition to the muscle. The blood flow measured by the total haemoglobin concentration under venous occlusion will be substantially more representative of blood flow in the muscle. It is therefore not surprising that it gives substantially higher absolute values than those obtained from venous occlusion, strain-gauge plethysmography.

Previous studies have shown skin blood flow to have a minimal affect on the NIR signal at optode separations above a few mm (Mancini *et al.*, 1994; Hampson & Piantadosi, 1988). Chapter 3 showed that muscle blood flow didn't generally change in the forearm, with changing leg workload (Fig. 3.11). This present study however, shows an increase in blood flow in the values taken immediately after exercise (BF2) only from the 60% leg exercise intensity bouts and onwards (Fig. 6.11). This is consistent with the pattern shown in the resting arm in the initial study (Fig. 3.10), attributed to rising skin blood flow. It suggests skin flow has a greater contribution to the total haemoglobin signal in the present study, than expected from the literature. Different algorithms and methods of determining the changes from NIRS may be the cause of this difference. Mancini *et al.* (1994) only used two wavelengths of near infrared light at 760 nm and 800 nm, and showed the absorbance at these wavelengths unaffected by changing skin blood flow. Hampson & Piantadosi (1988) used 4 wavelengths of NIR light, in a similar conformation to that of the NIRO-500 used in the present study. However, a comparison of the performance of several published tissue near infrared spectroscopy algorithms (Matcher *et al.*, 1995) found that that used by Hampson & Piantadosi significantly under-estimated changes in HbO_2 when compared to data from a continuous wavelength CCD-based tissue spectrophotometer, while the algorithm used in the NIRO-500 agreed well with the CCD-based spectrophotometer. This then raised questions over the accuracy of the Piantadosi algorithm.

At the 90% leg exercise intensity, as the arm workload increases, the forearm blood supply seems to become compromised. At the 1 kg workload, the arm seems unable to respond with an increase in flow of similar magnitude as that seen at other leg intensities (Fig. 6.8). At the maximum "2" kg workload, the exercising arm blood flow seems somewhat, although not significantly, reduced (Fig. 6.9). During this maximum bout, the blood flow increases from the value measured during exercise, to that taken after exercise ($p = 0.12$, Fig. 6.12). Blood flow to the exercising arm seems to become compromised in the maximal bouts of exercise, but probably the supine nature of the experiment (discussed in Chapter 3) again prevented this 'stealing' from becoming clearly significant.

Optode Positioning

The optodes were affixed to the volar surface of the forearm, over the belly of the flexor carpi radialis muscle. Effects due to movement were cancelled out by rigorously controlling the movement as previously discussed, and then averaging the data (Chapter 5). Changes due to increasing hyperaemia in the forearm could, however, have an effect. In 1953, Whitney described how under conditions of venous occlusion the rate of increase in forearm circumference correlates with the rate of inflow of blood. Little effect on forearm geometry may be expected however, in the longitudinal direction of the forearm. When attached parallel to the long axis of the forearm therefore, the optode position relative to one another was anticipated not to vary. This was supported by the results of the NIR signals

under venous occlusion when measuring blood flow. The changes in forearm geometry during these periods of venous occlusion will be many times greater than any occurring due to exercise hyperaemia. Any change in optode geometry would allow more or less light to be continuously detected by the receiving optode causing a consistent increase or decrease in the NIR signal. The lack of any regular change in the cytochrome oxidase NIRS signal during periods of venous occlusion, which would not be expected to change, supports this assumption. When placed longitudinally on the forearm, any affect on optode geometry due to hyperaemic response seems negligible. The absence of consistent CtOx changes is in marked contrast of course, to the total haemoglobin (Hb + HbO_2) situation - a fact which provides further evidence against the occurrence of significant cross-talk between their signals.

Deoxyhaemoglobin, Oxyhaemoglobin

The rates of deoxyhaemoglobin and oxyhaemoglobin concentration change were monitored in the forearm throughout the 4 minute exercise periods, and were split into those during the leg-only period (Hb_{leg}, HbO_{2leg}), and those from the arm-only or leg+arm period (Hb_{arm}, HbO_{2arm}).

Leg-Only Exercise

During the leg-only period, very little change was seen in Hb values in the forearm during the 30%, 60%, and 30%(2) bouts (Fig. 6.14). They were always slightly positive however. This is perhaps indicative of a slightly elevated oxygen uptake by the resting muscle of the forearm in anticipation of exercise. Above rates of about 100 bpm, the heart becomes regulated by sympathetic activation. This point coincides with the first significant leakage of noradrenaline from sympathetic nerve endings into the circulating blood plasma (Rowell, 1993b). Blood flow in the human body is reduced by increased sympathetic nervous outflow. The present experiments give evidence of some vasoconstriction in the resting forearm during leg-only exercise periods, because there is a greater fall in HbO_{2leg} (see below) than rise in Hb_{leg}. This could coincide with the spill-over of noradrenaline from the active leg muscles, or be due to increased sympathetic discharge to the resting muscles, increasing peripheral resistance and redistributing the blood volume to the active areas. An increase in noradrenaline has also been shown to cause a slight increase in whole body oxygen uptake (Weiβ *et al.*, 1988). Noradrenaline may have the dual action in the present study of slightly reducing resting arm blood flow, and slightly increasing oxygen uptake. This would cause the Hb_{leg} changes to be slightly greater than zero.

The falling rates of HbO_{2leg} concentration changes in the forearm during the same period of easy to moderate leg exercise are greater than the rising rate of Hb_{leg} concentration changes (illustrated on Fig. 6.2, and compare Figs. 6.14 and 6.15).

This is consistent with a redistribution of the fresh oxygenated blood supply from the arm to the exercising legs. Less HbO_2 will be supplied to the forearm, and coupled with the slight increase in oxygen extraction, will combine to give the greater HbO_{2leg} fall compared to Hb_{leg} rise.

The extra Hb increase and HbO_2 fall detected in the forearm during the 90% intensity leg-only bouts may be associated with exercise-induced hypoxaemia occurring in these intensive stages (Whipp, 1996; Katayama & Miyachi, 1996, Figs. 6.14, 6.15). An increase in muscle temperature and fall in muscle pH may also lead to a mild rightward shift in the oxyhaemoglobin dissociation curve causing a greater oxyhaemoglobin desaturation. The blood diversion effect also seems graded with leg intensity, which would add to the HbO_{2leg} fall during the 90% bouts. The HbO_{2leg} fall during the early stages of the 30%(2) bouts is significantly smaller than in the later bouts (Fig. 6.15). The much greater level of vasodilation and greater skin blood flow in the early 30%(2) stages shown in Chapter 3, and the large excess of arterial (i.e. oxygenated) blood available now that the legs are only exercising at 30% of their maximum again, will cause a large increase in forearm blood flow (and HbO_2 supply) during exercise, and reduce the HbO_2 change due to exercise. The still elevated heart rate (Fig. 6.3) and blood flow (Fig. 6.8) in these early 30%(2) stages is consistent with a greater cardiac output providing the greater blood supply.

Arm-Only, and Leg+Arm Exercise

During the arm-only and leg+arm exercise periods, the rate of Hb increase rose significantly with arm workload (Fig. 6.16). The increase in energy requirement and oxygen demand from the forearm muscle with increasing workload will cause this rise. The majority of the Hb_{arm} change occurred in the first 30 seconds of exercise (Fig. 6.17), suggesting that the forearm vasculature quickly adapted to the oxygen demand upon it. Very little Hb_{arm} change occurred in the subsequent 1.5 minutes of exercise (Fig. 6.19).

The HbO_{2arm} change fell significantly with increasing arm workload, but was not as clear-cut as the Hb_{arm} changes (Fig. 6.17). The changes in HbO_{2arm} were also significantly smaller than the Hb_{arm} changes (compare Figs. 6.15 and 6.16). This apparent mismatch has two causes. A greater increase in Hb_{arm} than decrease in HbO_{2arm} during exercise has been observed previously in both dynamic (Chapter 5) and isometric exercise (Chapter 4). An increase in Hb must come from an equal decrease in HbO_2, but the HbO_2 decrease can then be offset by further increases in fresh oxygenated blood supply, including that to areas monitored by the optodes but not actively involved in oxygen extraction and utilisation.

The lack of a regular pattern in the HbO_{2arm} changes could also be caused by different responses to exercise at different times during the 2 minute period of analysis. As with the Hb_{arm} changes, the HbO_{2arm} changes largely occur in the first 30 seconds (Fig. 6.20). In this period, the magnitudes of the Hb_{arm} and HbO_{2arm}

changes were much closer (compare Figs. 6.18 and 6.20). However, in the last 1.5 minutes, when there is very little Hb_{arm} change, the HbO_{2arm} values increase significantly in the moderate and hard arm exercise bouts offsetting the rate of charge seen overall (Fig. 6.21). This increase in HbO_2 is likely to be caused by increases in blood flow to areas other than those actively engaged in exercise, as there would be no change in the HbO_2 utilisation/Hb generation relationship.

The HbO_{2arm} fall, and the Hb_{arm} rise (Figs. 6.16, 6.17) seems to be smallest in the 90% leg intensity bouts (90% x 1kg, 90% x "2" kg) compared to the other bouts at 1 kg and "2" kg arm intensity. This is despite the possibility of significant exercise-induced hypoxemia occurring (seen clearly in the leg-only periods). This pattern is even repeated in the first 30 seconds, when most of the change in the Hb_{arm} and HbO_{2arm} occur (Figs. 6.18, 6.20). The energy demand for the arm exercise will not change with changing leg activity. As discussed earlier, the blood flow to the exercising arm has some evidence of being reduced in the most intensive bouts of exercise (Figs. 6.8, 6.9). This all seems consistent with an oxygen limiting situation. However, in Chapter 3, despite a possible reduced blood supply, the exercising muscle exhibited zero lactate movement between the blood and the muscle, or net uptake at these most intensive stages of exercise. If the lactate is then oxidised, and used as a fuel for energy generation (Jorfeldt, 1970; Hubbard, 1977; Chin *et al.*, 1991), it can only be metabolised aerobically. Connett *et al.* (1984) showed the muscle pO_2 to fall during maximal contractions, but to fail to reach limiting levels. Any shortfall in oxygen delivery during intense exercise in the present experiment must be made up from the muscle myoglobin

oxygen stores, reducing muscle pO_2. These stores are then replaced as part of the oxygen debt in the period of extended hyperaemia following the intense bouts of exercise, and in the subsequent 30%(2) bouts.

Belardinelli *et al.* (1995) showed a decrease in oxyhaemoglobin and oxymyoglobin saturation in the latter stages of 6 minute exercise bouts. This was in the vastus lateralis muscle during cycling, but was only at exercise intensities above the lactic acidosis threshold. This they concluded was due to the progressive decline in muscle oxygen saturation in the contracting muscles above the acidosis threshold, consistent with the lactic acidosis threshold being the point of oxygen limitation in the muscle. This is consistent with the mild myoglobin desaturation suggested above. However, it seems they may have overestimated the contribution from myoglobin.

Belardinelli *et al.* (1995) estimated the relative saturation of the tissue being monitored from the difference in the absorbance of light at 760 nm and 850 nm. They stated the value of Δ(760 - 850 nm) to be relatively independent of changes in total HbO_2 and MbO_2. This thesis has shown that the changes in HbO_2 and Hb are by no means necessarily coincident. NIR light at 760 nm picks up largely the changes in Hb, and light at 850 nm largely the changes in HbO_2 (Cope, 1991; Elwell, 1995). If blood flow, and therefore HbO_2 supply increase in the latter part of an exercise bout, the absorbance of light at 850 nm will increase. Hb production is unlikely to change (as seen in this chapter in the latter 1.5 minutes of the 2 minute periods of arm-only or leg+arm exercise). This will result in Δ(760 -

850 nm) showing a gradual fall, but caused by an increase in oxygenated blood supply, rather than a decrease in saturation.

Cytochrome Oxidase Oxidation State

The rate of change in the redox state of cytochrome oxidase was also monitored in the exercising forearm throughout the 4 minute exercise periods, and again was split into the leg-only period ($CtOx_{leg}$), and the arm-only or leg+arm period ($CtOx_{arm}$).

Leg-Only Exercise

During the leg-only exercise period, there is little change in the cytochrome oxidase redox state (Figs. 6.2, 6.22). However, during the third bout of 90% leg cycling intensity, when in Chapter 3 the arterial lactate concentration was highest and the blood and muscle pH therefore may be assumed to be lowest, the cytochrome oxidase redox state was just significantly more oxidised. The extreme conditions generated by the repeated bouts of 90% of maximum leg cycling may be inhibiting the enzymes of the ETC causing a reduction in the supply of electrons to cytochrome oxidase, causing the same effect as that outlined in Chapter 4, and the enzyme to become more oxidised. The following 30% (2) bout causes the most negative redox potential, which then returns back to zero change

with repeated 30%(2) bouts. Restoration of the 'oxygen debt' created during the 90% stages could be responsible for this effect. The excess blood flow and oxygen supply to the forearm muscle in the leg-only period of the early 30%(2) bouts is consistent with a greater flow of electrons down the ETC, causing the cytochrome oxidase to become more reduced. This effect will fall as the body recovers, and the oxygen debt is repaid, signalled by the gradual return of the cytochrome oxidase redox state to zero change by the third leg-only period.

Arm-Only, and Leg+Arm Exercise

During the arm-only and leg+arm periods, the cytochrome oxidase enzyme becomes more reduced with increasing arm intensity (Fig. 6.23). Similar to Chapter 5, the easy, 0 kg arm workloads show no effect. The 1 kg and "2" kg bouts are not significantly different, but both become significantly further reduced compared to the 0 kg bouts. There is however, no significant effect due to increasing leg intensity. In Chapter 5, the majority of the change in the cytochrome oxidase redox state occurred in the first 30 seconds of the 2 minute exercise period, with very little change in the latter 1.5 minutes (Fig. 5.18). A similar situation was seen in the present study (Figs. 6.24, 6.25). The two periods of analysis both have the same general pattern of becoming more reduced as arm workload increases. However, in the latter 1.5 minutes, particularly at the higher workloads, the cytochrome oxidase shows generally smaller rates of change, and also becomes slightly more oxidised, or less reduced than it had been in the first

30 seconds. This can be argued to be due to the changing intramuscular conditions causing a reduction in electron supply down the ETC to cytochrome oxidase, but the effect is at best equivocal.

The curtailed hyperaemia and hence somewhat reduced oxygen supply to the forearm during the moderate and hard arm workloads in the 90% leg intensity bouts seems therefore to have little significant effect on the cytochrome oxidase redox state. If the muscle never becomes truly oxygen limited, electrons reducing the cytochrome oxidase will always be able to go on and reduce oxygen to water. The muscle oxygen tension may fall to levels nearer to limiting values, but still be adequate for aerobic metabolism. The 2 minute leg+arm period may not be sufficient to achieve oxygen limitation. The extra fall in oxygen tension may only serve to reduce myoglobin oxygenation slightly and increase the oxygen debt incurred, and contribute to the elevated blood flow and extended hyperaemic effect seen after the most intensive bouts.

The energy demand will remain constant for the three arm workloads throughout the study. The increasing leg workloads will however cause the arterial lactate concentration to rise, and pH to fall. These are two of the factors that affect the mitochondrial environment in the 'closed' system of the isometrically contracting, occluded muscle (Chapter 4). There they were proposed as causing the reduction of supply of electrons down the ETC to cytochrome oxidase, resulting in the enzyme becoming more oxidised. Despite arterial lactate concentrations likely to reach over 7 $mmol.l^{-1}$ in the present study (compare Chapter 3), only the

exercising arm prior to exercise, during the initial leg-only period shows any effect (Fig. 6.2, 6.22). During the arm exercise period, elevated arterial lactate concentrations may cause net lactate uptake by the arm. A substantial part of this is likely to be immediately oxidised, and contribute electrons to the ETC. There will be no change in the energy demand with increasing leg exercise intensity. If the blood lactate taken up by the exercising muscle is metabolised instead of glucose to supply the energy demand (Richter *et al.*, 1988), there will be no change in the generation of reducing equivalents, and supply of electrons to the ETC. It would therefore not alter the cytochrome oxidase response. The conditions themselves may cause a slight fall in the rate of reduction/rise in rate of oxidation, but do not seem to be sufficient to cause a significant effect on the flux of electrons through the cytochrome oxidase enzyme complex. This then results in the lack of a significant affect on the muscle cytochrome oxidase redox state during exercise.

Chapter 7

CONCLUSIONS, AND FUTURE WORK

CONCLUSIONS

Set against the hypotheses of pages 33-33c, the findings of this thesis may be summarised as follows:

- Only a small amount of blood stealing could be detected, and it did not correlate with lactate production, as hypothesised for Chapter 3. On the contrary, as arterial blood lactate concentrations increased caused by the exercising leg muscles, a forearm muscle exercising at intensities up to maximum switched from net production to net uptake. This indicated that lactate flux was dependant on its concentration gradient across the muscle membrane, with the further implication that lactate production could not be linked to oxygen supply.

- Cytochrome oxidase, the terminal electron acceptor of the Electron Transport Chain (ETC) and the only point that makes direct use of oxygen in energy generation, was shown to become more oxidised during occluded isometric exercise. This was attributed to the build-up of metabolites from energy metabolism affecting the mitochondrial environment, diminishing the flux of electrons down the ETC and through the cytochrome oxidase enzyme complex. The enzyme would then receive less electrons and become more oxidised. The hypothesis that oxygen supply to the forearm muscle would not become limiting in isometric exercise was therefore upheld (Chapter 4).

- However, the cytochrome oxidase enzyme was shown to become more reduced during dymanic exercise. This was attributed to the high aerobic energy demand of dynamic exercise increasing the electron flux down the ETC and through the enzyme complex. The enzyme would then receive more electrons and become more reduced - contrary to the hypothesis of Chapter 5.

- The dynamic exercise of Chapter 6 also produced some conflict with the hypothesis, in that the oxidation of the forearm muscle declined in some exercise conditions. However, the redox state did not vary consistently in relation to conditions of net lactate output, and net lactate uptake. This supported the concept that lactate production was not oxygen related, but instead suggested that when lactate was taken up it was being metabolised and used as a carbohydrate energy source for energy generation.

- The key positions reached are:

1. That lactate is a metabolizable intermediate, not an end-point product of a metabolic pathway which is obliged to operate when the oxygen supply is inadequate.

2. That the redox state of cytochrome oxidase, the only point in energy generation that actually makes direct use of oxygen, seems to be determined by the flux of electrons through the enzyme complex, and not related to the

supply of oxygen, which therefore must be presumed to be present in excess in normal physiological conditions.

FUTURE WORK

The work described in this thesis suggests several areas of future investigation.

Confirmation Experiment

An experiment to confirm the mechanisms proposed above could be to perform dynamic exercise initially with free blood flow to the exercising muscle, then continuing the exercise but under occlusion. If the models in this thesis are correct, the cytochrome oxidase will initially become more reduced, then as the occlusion traps the metabolites of energy metabolism the enzyme will become more oxidised.

NIRS at Exhaustion

In exercise of short enough duration to prevent muscle glycogen depletion, if oxygen never becomes limiting what happens to the cytochrome oxidase oxidation state at exhaustion? Work in this area has already been presented in abstract form

(Ward *et al.*, 1994; Whipp & Ward, 1994; Ward & Whipp, 1997). In maximal, isometric quadriceps extension contractions, these authors found similar changes to those presented in this thesis, but also that the noisy nature of the cytochrome oxidase signal made not all the results consistent. They concluded from this that the unusual, and inconsistent response of the cytochrome oxidase raises questions regarding the validity of NIRS, as currently available, for assessing the cytochrome oxidase redox state. However, it is perhaps permissible to note that the Whipp group are one of the groups still maintaining oxygen limitation as the cause of lactate production. This may contribute to their conclusions, and to bringing a disposition different from my own, to the interpretation of similar data.

If oxygen never reaches limiting levels in exercising muscle, then exhaustion could be mediated by the inability to regenerate adequate ATP due to a reduced efficiency of the enzymes of energy generation. If this is the case, the cytochrome oxidase will become equally oxidised at exhaustion, regardless of the mode of exercise. By carrying out maximal exercise tests in conditions of normoxia, it is anticipated that initially the cytochrome oxidase would become reduced, and then become less reduced/more oxidised as the inhibitory effect of the muscle metabolites builds up towards exhaustion. If the same test is then carried out in conditions of hypoxia (simulating altitude), exhaution would occur sooner, and the cytochrome oxidase would be expected to reach the same level of oxidation at exhaustion, only to achieve it quicker. This could form the basis of a future research project.

Mitochondrial Volume

Training induced increases in $\dot{V}O_{2max}$ have recently been shown to correlate with increases in mitochondrial volume and electron transport capacity, opinion perhaps beginning to swing back again to peripheral (as against central) limitations of oxygen uptake (Robinson *et al.*, 1994). Greater aerobic capacity will cause greater electron flux down the electron transport chain, and through cytochrome oxidase. If peripheral factors do indeed limit oxygen uptake, could the maximum level of reduction of the cytochrome oxidase enzyme be used as an indicator of training state?

Dichloroacetate (DCA)

A new study looking at the metabolic response of the rest-work transition found that dichloroacetate (DCA) administration increased pyruvate dehydrogenase complex activation, and also resulted in acetylation of 80% of the free carnitine pool (Timmons *et al.*, 1997). This led to a much greater oxidative contribution to the energy supply, accompanied by a reduced reliance on PCr to maintain ATP availability, and reduced glycogen utilisation and lactate formation. By increasing the speed of onset of oxidative phosphorylation, faster regeneration of reducing equivalents would occur. NIRS could be readily applied to this situation. According to the mechanisms of action of cytochrome oxidase proposed in this thesis, the DCA treated muscle would experience a more rapid, and possibly a

greater reduction in the oxidation state of cytochrome oxidase. This again could form part of a future research project.

Lactate Uptake

Further studies can also be carried out involving the blood lactate uptake seen in this thesis. Sufferers of McArdle's syndrome are unable to use glycogen to produce lactic acid due to the absence of glycogen phosphorylase. Increasing the availability of oxidisable substrate (e.g. by glucose infusion) increases the maximal oxygen uptake, and partially corrects for the muscle phosphorylase deficiency (Lewis & Haller, 1986). As lactate is a more broken down form of glucose, then will McArdle's syndrome patients be able to use lactate as a carbohydrate source for more rapid energy generation, if it is infused into the blood perfusing the muscle?

Lactate Uptake and Dichloroacetate

The potential of DCA administration affecting the rate of uptake and utilisation of lactate also merits further investigation. If DCA increases the activation of the pyruvate dehydrogenase complex, then it can be postulated that it will also increase the utilisation of lactic acid as an energy source. However, the clarity of this [rediction is compromised by reports that DCA also increases the availability

of free fatty acids. If these were used preferentially, they could suppress lactate uptake. Preliminary work in this area in animals is discussed by Gladden (1989). Clarifying experiments along his lines would be required before the complexities involved in studying vigorous exercise in DCA-treated subjects with Near Infrared Spectroscopy could judiciously be embarked upon.

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Appendix 1

GREATER GLASGOW HEALTH BOARD

THE WEST ETHICAL COMMITTEE

FORM OF CONSENT FOR PATIENTS/VOLUNTEERS IN CLINICAL RESEARCH PROJECT

Brief Title of Project

INVESTIGATION INTO THE EFFECT OF EXERCISING SMALL AND LARGE MUSCLE GROUPS ON THE BLOOD FLOW AND LACTATE THRESHOLD OF THE FOREARM.

Patient's Summary (Purpose of study, nature of procedure, discomfort and possible risks in terms which the patient or volunteer can understand.)

Before being accepted as a subject in this investigation, you will be required to undergo an appropriate medical examination. This may be done by your own G.P., or by a doctor arranged by the experimenters-whichever you prefer. As a subject, you would be required to participate in three different phases of work. The first phase will involve continuous cycling at increasing levels of severity, while your power output, heart rate and expired air are monitored. This will give a value of the maximum rate at which your body can take up oxygen (a commonly used measure of fitness) and your maximum work rate on a cycle ergometer. The test usually lasts less than 40 minutes, and qualified medical personnel will be close at hand at all times, against the unlikely event of trouble.

The second phase will involve a simple wrist curl exercise to determine the work intensity at which lactic acid starts accumulating in the forearm (the "lactate threshold"). Medically qualified personnel will take blood samples from both a pricked finger and a vein at your elbow immediately following each period of exercise. The blood will be taken from the vein by either a small needle or a fine flexible tube - your preference will be taken into account in choosing between these. These periods will last for 2 minutes each, with 5 minute recoveries between each period. Blood flow rate will also be measured immediately after each level of exercise; the technique requires a cuff to be very lightly inflated round each arm. This stage will last no longer than 1 hour, and will be at least two, and not more than five days after the previous phase.

In phase three, you will be required to cycle at each of three predetermined levels (30%, 60%, or 90% of the maximum intensity achieved in phase one) whilst simultaneously carrying out some of the wrist curl exercises of phase two. Blood sampling and blood flow rate measurements will be identical to those of phase two. Again, this session will be arranged 2-5 days after that of stage 2.

In order to complete the entire investigation, you would thus be required to attend three times, over a period of 4-10 days. However, you would be free to end your participation in this investigation at any time, if you so wished. Your G.P.'s name and address would also be required, so that they could be informed of your participation in this investigation.

Consent

I, ... of .....
give my consent to the research procedures described above, the nature, purpose and possible consequences of which have been described to me by .....

Signed ..... Date .....

Witness .....

VOLUNTEER SUBJECT'S INFORMATION AND CONSENT FORM

TITLE

INVESTIGATION INTO THE EFFECT OF EXERCISING THE FOREARM ON MUSCLE OXYGENATION LEVELS, MEASURED BY NEAR INFRARED SPECTROSCOPY, AND LACTATE CONCENTRATION, UNDER NORMOXIA, HYPEROXIA, AND HYPOXIA.

PEOPLE CONDUCTING THE RESEARCH

N. C. Spurway, M.A., Ph.D., Senior Lecturer in Physiology; Course Organiser, Physiology & Sports Science Degree.
J. L. Bradley, B.Sc., Research Student, Exercise Physiology.

SUMMARY

As a subject in this investigation you will be required to attend three testing sessions. In each testing session, a qualified person will insert a cannula into a vein on the upper surface of your dominant (or preferred) forearm, and you will be asked to perform two periods of handgrip exercise whilst breathing air containing either a normal, an elevated or a reduced level of oxygen. The hand grip periods are of 2 minutes duration. The air being breathed will be of medical standard. The elevated oxygen air mixture will contain 100% oxygen, and the reduced oxygen air mixture 12% (equivalent to air at about 4000 meters), compared to 21% in normal sea level (room) air. You will breath the air mixtures for 1.5/2.5 minutes prior to the hand grip exercise, during the exercise, and 1/1.5 minute after the exercise - 4.5/6 minutes in all. In the second period of each testing session, a cuff will be inflated around your exercising arm 1 minute prior to the exercise to stop blood flow, and released 30 seconds after the end of the grip. During each period, blood samples will be taken by qualified personnel from your forearm, and blood pressure and muscle oxygenation levels will be measured non-invasively.
Except for a possible slight dizzy, and/or nauseous feeling, which occasionally occurs, no side-effects should be experienced due to the experiment. However, you are free to end your participation in this experiment at any time.

CONSENT

I,_____ of _____
give my consent to the research procedures described above, the nature, purpose and possible consequences of which have been described to me by _____
_____.

SIGNED_____ DATE_____

Appendix 2

Two NIRO-500 spectrophotometers were used in the preparation of this thesis. The specific extinction coefficients for HbO_2, Hb, and CtOx at the 4 specific wavelengths were:

Chapter 4, Experiment 1:

Wavelength	775	827	852	905
Hb	1.2474	0.7338	0.7147	0.7169
HbO_2	0.7034	0.9714	1.0606	1.0782
CtOx	1.9940	2.1969	2.0728	1.4254

All other NIRS studies:

Wavelength	775	825	845	904
Hb	1.2474	0.7373	0.7178	0.7186
HbO_2	0.7034	0.9641	1.0391	1.0804
CtOx	1.9940	2.2121	2.1051	1.4357